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INCREASE OF THE ADAPTIVE RANGE OF THE GENUS DELPHINIUM

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INTRODUCTION

The genus Delphinium is closely allied to *Aconitum*. Species of both genera resemble each other closely in vegetative habit, and certain Eurasian species of both approach each other in details of floral structure. Both genera are similar cytologically. The chromosome number is the same in both. The karyotype of *Delphinium* is apparently similar throughout the genus, except for ploidy (Lewis *et al.*, 1951). But Schafer and La Cour (1934) have shown that the karyotype of *Aconitum* is variable from species to species, and within species, in respect of chromosome length and spindle attachment. Of particular interest here is the fact that a choice of certain chromosomes from the variants shown by them (p. 702, chromosomes ABDEIJKM) would provide a genome scarcely distinguishable from that common to *Dephinium* species. These similarities of structure and karyotype strongly suggest therefore that both genera are divergent members of the same phylad.[1]

This phylad is widespread. Both genera are holarctic and are primarily constituents of present day elements of the Arcto-Tertiary Flora. The generally mesic character of both genera and the historical relations of the communities with which they are associated indicate that they have been a part of this flora for a long period and have become accommodated to the progressive changes of environment within it. Although both genera may occur in the same communities, *Aconitum* has retained a more mesic character than *Delphinium*, which has often become adapted to more stringent environments as these have developed during later Cenozoic time. Some of its species, such as those with which we are

[1] The considerable degree of interfertility which exists between even sympatric *Delphinium* species, as we shall show in a subsequent paper, and between those geographically far removed, such as certain American and Eurasian species, suggests the possibility of interfertility between *Delphinium* and certain chromosome races of *Aconitum*. We have attempted to grow Himalayan species of *Delphinium* and American species of *Aconitum* with the intention of ascertaining whether such interfertility exists, but have been unable to bring either to flower under the ordinary greenhouse conditions of Los Angeles. Investigation along this line in a more suitable location might result in a demonstration of the origin of one well marked genus from another, and the possible means by which it has occurred.

concerned, have entered into communities of the Mediterranean and desert climates of Eurasia and North America.

The subject of this paper is a species group of *Delphinium* in California which is associated with communities of this sort. These communities range from the relatively mesic oak woodland of coastal California to the xeric bush communities of the Colorado desert. They have been segregated since later Pliocene time from the earlier Madro-Tertiary Flora of neotropical origin (Axelrod, 1948). The purpose of our discussion is to examine the adaptive character of this species group, and the means which have enabled it to explore and occupy an ever widening ecological periphery as the later Cenozoic trend of climate has developed in this area. The means are not necessarily confined to *Delphinium*, but may also be illustrative of those evolved by other phylads which have had their origin in the Arcto-Tertiary Flora and which are now included in these communities.

The Adaptive Response to Weather

The examples which we shall use are drawn from seven closely related species which we have studied intensively both in our experimental garden and in the field. Each species has a different adaptive norm which is perhaps best indicated by the vegetation type with which it is primarily associated.

D. gypsophilum Ewan	Grassland bordering the San Joaquin Valley
D. hansenii Greene	Oak-digger pine woodland, Sierra Nevada foothills
D. hesperium Gray	Oak-digger pine woodland, Coast Ranges
D. parishii Gray	Desert scrub, Mojave and Colorado deserts
D. parryi Gray	Chaparral, South Coast Ranges, Southern California
D. recurvatum Greene	Saline flats, San Joaquin Valley
D. variegatum T. & G.	Oak-digger pine woodland, Sierra Nevada foothill and the Coast Ranges

The geographic ranges of these species generally overlap to some extent or other, and two or more species may sometimes occur at the same site. But the sites and associates of each species are generally different when they occur in the same territory and are generally characteristic of each. Observation of the slope, the soil type, and certain conspicuous components of the vegetation often may lead one to a colony even though it is not in flower at the time and, hence, not readily found.

The weather to which these species respond is highly diversified locally and fluctuates greatly both in the absolute values of its variables and also in their effectiveness. There exists, first of all, a general gradient of temperatures and rainfall from north to south and from the coast to the desert interior. This gradient is irregular because of the broken terrain, and differences of considerable magnitude may exist between localities only a few miles apart. These differences are increased because of the variation from year to year of the factors concerned, particularly of rainfall, both in total amount and in seasonal spread. The yearly total at Los Angeles, for example, has ranged in recent years from as much as 40 inches to as little as 7 inches. The effectiveness of whatever amount falls is partly determined by the seasonal spread in relation to the prevailing temperatures. Most rain falls usually in January, but the peak may occur exceptionally as early as November or as late as May. During some years a large proportion, as much as 65 per cent, may fall during one month, or even in the course of one storm. In this case the run-off is correspondingly large and the effectiveness reduced. The period of drought during which no rain occurs may last from March until December. In

other years rain may fall in measurable amounts during almost every month of the year, but its effectiveness may be greatly reduced because of low monthly totals, and may vary locally because of differences of soil types.

These fluctuations follow trends which are sustained for variable periods and give the impression of a crude cycle (Lynch, 1948). Departure from the norm is least in the coastal areas and greatest in the desert mountains. The general character of the trend for southern California is illustrated by a computation of rainfall for San Bernardino since 1780, compiled by Prof. M. R. Huberty. His graph represents the departures above and below "normal," represented as a series of 10 year moving averages (Fig. 1). For a period of about 50 years, approximately from 1845 to 1895, the fluctuations of rainfall were more or less equally spaced over short intervals. The climate was correspondingly equable. Prior to this and subsequently, the trend of departure has been much more accentuated by periods of alternating wetness and drought. Each phase has lasted for 10–20 years, has gradually reached a maximum of rainfall, or a minimum, and then has passed gradually into the alternate phase of that "cycle." For example, rainfall was below normal, on the average, for about 25 years from 1785–1810, the minimum averages falling to 80 per cent of normal; it was on the average above normal for the following 12 years, reaching a maximum of about 130 per cent, and dropped again during the next decade to about 75 per cent of normal.

Hence, the climate we are concerned with is not only an arid one but is also marked by fluctuations of weather of considerable amplitude, both from place to place and from year to year and at the same time over longer periods during which drought may be severe and sustained.

The adaptive response of plants, and indeed of many organisms, to arid and fluctuating environments such as these appears to be chiefly by means of varying degrees and kinds of dormancy. Numerous annuals such as *Layia munzii* (Clausen, 1951) and *Linanthus parryae* (Epling unpub.) exist only as seeds for several months of the year; growth is confined to short periods and may be held in abeyance for several years. Perennials, such as *Salvia* may lose a considerable amount of their foliage during the period of drought, resembling thus certain insects which respond to unfavorable periods by an arrested development. Our species of *Delphinium* are able to persist because of a dormancy of greater fixity which is parallel in effect with the fixed diapause of many insects. A population is accordingly able to persist in a dormant condition through a sustained trend of weather during which it would otherwise perish.

Dormancy is expressed in *Delphinium* by the following yearly cycle. The primary roots are clustered around a small

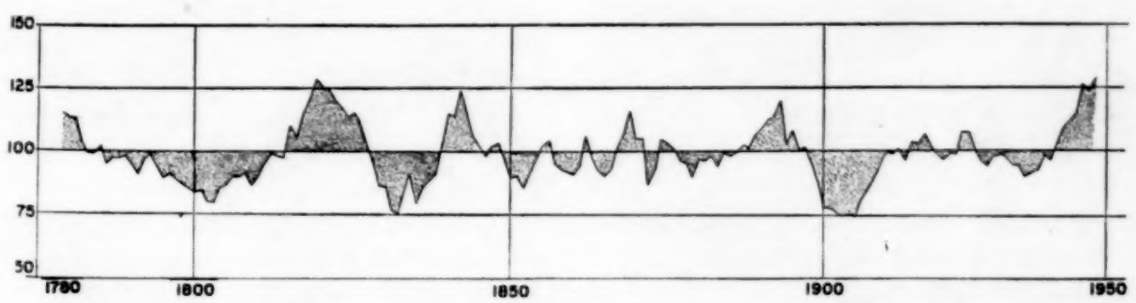

FIG. 1. Estimated rainfall of San Bernardino, California, for the period indicated, measured as a percentage of normal and based on a 10-year moving average. Compiled by M. R. Huberty.

crown of stem and are shallowly seated in the top soil. During the summer they are dry, brittle and seemingly dead. After the first penetrating rains of late autumn and early winter, they absorb water, become fleshy, and send out secondary roots to replace those which have long since withered. If the events of weather follow thereafter in favorable sequence each plant develops a rosette of leaves from a bud or buds on the crown, followed in spring (from late February to April or May, depending on the species and site) by a flowering stalk. After maturing its fruit and scattering its seeds, about a month after flowering, each plant again becomes dormant. Dormancy ordinarily lasts about six months.

Observation of large numbers of potted plants, grown under different conditions of cultivation, that is, out of doors, under lath, under screen, and under glass, together with field experience, indicates that the primary cause of dormancy is the water relation, probably expressed both as available water in the soil and by the saturation deficit of the air. Conditions for breaking dormancy appear to be the temperature and available moisture of the soil. There appears also to be a complex relation between the yearly course of development and the progression of seasonal changes of weather. Development of an individual to the completion of its reproductive phase apparently continues only within a narrow range of conditions. If these conditions are not present at any time during the growing season, dormancy follows; once initiated, it continues and growth is not resumed until the following season. Seedlings may become dormant shortly after expanding their cotyledons and yet may be viable and produce a rosette the following year. Older plants which have reached various degrees of the rosette stage may stop growing and the leaves wither. Or plants in bud, or even in full flower, may become dormant before the fruit is set; they may dry out so rapidly that the open flowers wither, and yet retain their form and remain in place on the infloresence. Even so, the vitality of the root is not affected, for if such plants are brought into the garden they will grow vigorously the following year.

We have observed this phenomenon repeatedly in the field. A colony consisting of hundreds of individuals in the rosette stage may produce few or no plants which flower. In March 1946, for example, we observed a colony of *D. parishii* near the foot of Banner Grade, San Diego County, which consisted of hundreds of vigorous rosettes. When it was revisited five weeks later in order to study the flowers and flowering habit, there was no sign whatsover above ground that any delphinium had grown there; the rosette leaves had completely withered and disappeared. Anyone searching the region during this season would have reported *D. parishii* absent from the area. Of further interest is the fact that this population completely failed to produce even rosettes in 1951.

If the conditions required for breaking dormancy do not occur, the plants concerned may remain dormant for an indefinite period. Or if dormancy is broken the plants need not flower. We know of populations which have not flowered for a decade. For example, *D. gypsophilum* flowered in great abundance in the spring of 1941 at a site one mile north of McKittrick. A few plants flowered in the spring of 1942, but none flowered from then until 1952. No rosettes, even, appeared from 1942 until the spring of 1950 and 1951, when a few were found. In the spring of 1952 flowering individuals were more abundant than in 1941. Comparison of the roots of such plants with roots grown in the garden for as long as 10 years show that they are not young plants but old ones which have persisted in dormancy for the periods concerned. To judge from the sizes of some roots it would seem that the life span of an individual can frequently be reckoned in decades.

Colonies of *Delphinium* may accordingly persist at a site for very long periods. Those we have studied appear to have changed very little for a decade except for the numbers in flower during a given season. Our observations concerned with pollen and seed dispersal and establishment, which follow later, also indicate that the total population of a colony changes very slowly or infrequently. Colonies vary greatly in size and are usually separated by at least several hundred yards. The total population of a colony can usually be estimated only during the most favorable seasons, because the number and spacing of the individuals actively growing may vary tremendously from year to year. A colony of *D. gypsophilum* near Coalinga stretched continuously for two miles and comprised tens of thousands of individuals in the spring of 1941. Revisited the following spring, it appeared then not as one colony but as a few scattered groups in moister soil along the shadier slopes of ravines. Each group consisted at most of tens of individuals. Or, to cite another example, what had seemed for several years to be a series of small adjacent colonies of tetraploid *D. hansenii* near Caliente, became one more or less continuous colony in the spring of 1950, numbering thousands of individuals (Fig. 2). But the colonies of diploid *D. hansenii* and *D. gypsophilum* in this same drainage

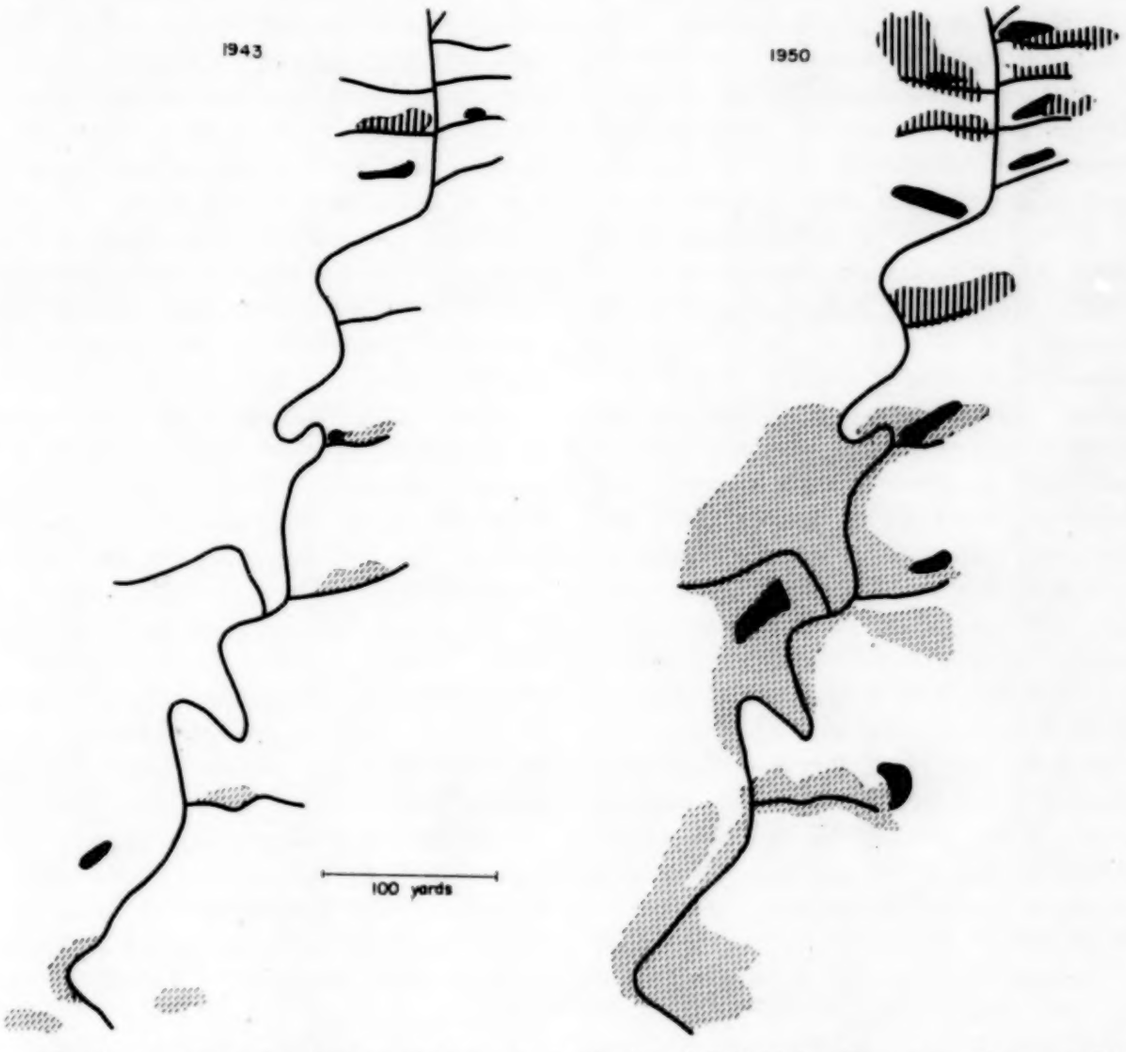

FIG. 2. Apparent distribution and extent of colonies of *Delphinium* in one small canyon in the years indicated. The solid patches represent *D. gypsophilum* (diploid); the broken areas *D. hansenii* (fine broken lines, tetraploid; solid perpendicular lines, diploid).

changed primarily in number and location of colonies appearing rather than in the extent of any one colony. It is of particular interest to note that not only were more than twice as many colonies of *D. gypsophilum* found in this small area in 1950 as compared to 1943, but that they did not necessarily occupy the same sites. The three colonies that were found to the left of the main drainage in 1950 were in places where no delphiniums were observed in 1943. It is possible that a few rosettes of *D. gypsophilum* may have been present at these spots on the earlier date, but were overlooked. However, the areas to the left of the drainage where two colonies had been specifically noted in 1943 were carefully examined in 1950 without finding a single representative of the species. The total population of a colony is accordingly difficult to estimate because of these differences of response. Considering the frequent limitations of site, we are inclined to believe that most colonies number several hundreds rather than thousands. Some, in fact, appear to be much smaller, and some individuals may be solitary.

Colonies ordinarily consist of only one species. The ranges of all the species treated here overlap in one way or another, and in these common areas two species, or even three, may occupy the same site. In this event they may or may not flower simultaneously each year. Two colonies along the Marsh Creek Road, Contra Costa County, are examples. One lies within lush oak woodland, not far from Mt. Diablo. The other lies at the thin margin of the woodland near Byron overlooking the arid San Joaquin valley. Both are mixed colonies of *D. variegatum* and *D. hesperium*. *D. parryi* also occurs at the latter site. *D. variegatum* and *D. hesperium* have flowered each year at the first site in varying numbers and proportions. The hillside at Byron was covered in 1942 by a mass of intermixed *D. hesperium* and *variegatum*, but none have seen since and we have found there only *D. parryi*, which is the most southerly in distribution of the three and is usually associated with the more xeric chaparral, rather than with woodland. A few individuals of this species were observed in 1944, two in 1947, one in 1949, and 32 in 1951. Similarly, a large colony of *D. hesperium* was found near the Pinnacles National Monument in both 1941 and 1942. When the site was revisited in 1946, only *D. parryi* was found growing.

We have studied this phenomenon systematically for six years following a transect along the highway south of Paicines for 24 miles. Both *D. hesperium* and *D. parryi* occur in this territory and sometimes form mixed colonies. The number and kind of individuals in each colony were recorded each year. The data are summarized in Figure 3 which gives a general, but incomplete idea of the composition of each colony, their spacing, and to a small extent the numbers of individuals found either in flower or in bud. A few colonies visited in 1941 primarily for transplants and herbarium records are also included because that year has proved to be the peak of the present trend of weather, until 1952.

These data confirm our observations of particular colonies such as those previously referred to and show the differences of yearly response on the part of colonies in the same region and at the same locality. Let us refer, for example, to the station 21.7 miles south of Paicines. Only *D. hesperium* flowered in 1941, when it was abundant, and again in 1949. Only *D. parryi* flowered there in 1946, 1947 and 1951. Neither species appeared in 1948 or 1950. In 1949, two individuals of *D. hesperium* appeared 0.2 miles north of this colony (and may be a part of it), but have not been seen since; in 1951 two appeared 0.2 miles south of the group of *D. parryi*, but had not been observed there before. In 1952 both species, together with a number of intermediates were present. At a colony 1.4 miles farther south, only *D. parryi*

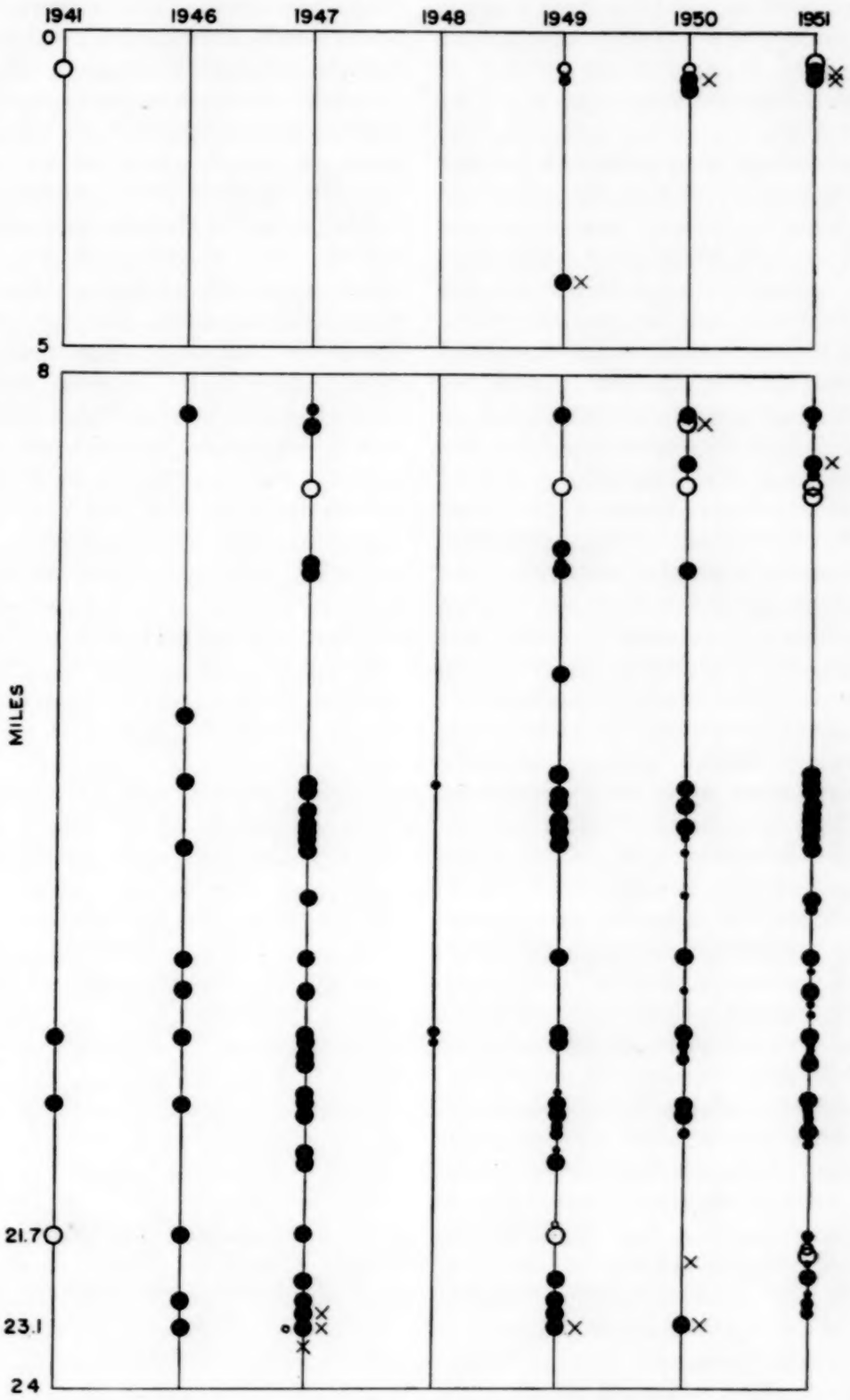

FIG. 3. Diagram of the apparent distribution of colonies of Delphinium for 24 miles south of Paicines in the years indicated. Solid circles represent *D. parryi*, open circles *D. hesperium pallescens*, and X hybrids. The smallest circles represent 1–3, intermediate 4–9 and the largest 10 or more individuals. Hybrids were fewer than 10 in all cases. Each symbol on the line represents a distinct colony even though the symbols overlap. Mixed colonies are shown by separate symbols at the same horizontal level.

was found in 1946 and 1952, both species, together with their hybrids, were found in 1947, only *D. parryi* and hybrids in 1949 and 1950, and no plants at all in 1948 and 1951.

Mixed colonies such as these hybridize to a greater extent than is suggested by Figure 3, as we know from transplant experiments. Hybrids may be present or even abundant in a colony but not flower, or flower only occasionally. Observation indicates further that they tend to complete the reproductive cycle in response to conditions different from those to which the parent species respond. We have followed, for example, a mixed colony of *D. hesperium* and *D. variegatum* on the Marsh Creek road near Mt. Diablo for a number of years. We know from experiment that the F_1's of these species are produced readily and that these F_1's set about 25 per cent viable seed in the garden; but we have found only an occasional hybrid in flower in the colony itself. When a series of plants were taken up in the rosette stage and potted, they flowered in a screenhouse the following spring. A much larger proportion was found to be hybrid in one degree or another, many more than was suspected from previous observation of the colony. At the same time, hybrids present in a colony may flower abundantly under some conditions, as in 1952 when they appeared in appreciable numbers in the Marsh Creek colony, although both species were less abundant than usual. A mixed colony of *D. gypsophilum* and *D. hansenii* near Caliente provides an example. In 1943 diligent search was made among hundreds of plants of the large population then in flower. Only an occasional hybrid was found. Intermediates between these types are readily recognized, as we know from extensive hybridizing experience with them. During subsequent years the populations were small and set very little seed. They were large again in 1950 and the canyon abounded in hybrids, their local frequencies being as high as 30–50 per cent. The maturity of the plants and the known conditions of drought meanwhile made it highly improbable that these frequencies were built up during this period by establishment of new hybrid individuals. It would seem much more probable that the conditions of 1950 were particularly favorable for flowering of the hybrids which appeared so abundantly in that year. Furthermore, the hybrids which flower in different years in a given colony, tend to be different individuals. This is shown by their relative positions, and by morphological differences. This fact has been clearly established by observation of an artificial colony in the Santa Monica Mountains. In the autumn of 1944 we sowed about 10,000 seeds of diverse origins in an area there of about 100 square feet. The seeds used were partly surplus seeds from various crosses, partly from open pollination of different species in the garden, and partly wild. About 75 vigorous seedlings came up the following spring. They proved to be of hybrid origin in many cases, and their parentage generally was identifiable. Their positions are known and the site has been inspected each year since. Following 1945, the number of rosettes and of flowering plants diminished year by year. None at all appeared in 1948 and it was supposed that the colony was lost. In 1949, 31 plants reappeared and in 1950 approximately the full number was restored. They are almost certainly the same individuals as those observed in 1945. Meanwhile, the weather has fluctuated from year to year in this region and we infer that the different genotypes which have broken dormancy, and those which have flowered, have done so because of differences of response to these differences of weather.

To establish this fact and to determine the extent to which different genotypes within the same species respond differently to differences of weather can be ascertained only by experiment under

controlled conditions. Whatever such differences of individual response may prove to be we are convinced by observation that a differential response of genotypes to weather exists within given colonies. During the phase of weather when conditions reach a certain optimum for a colony, large numbers of its component individuals may flower and set seed. During less favorable phases, only a small proportion are able to do so. Our observations suggest that the latter represent a component of the colony which has a different adaptive norm which fits it particularly for the conditions prevailing.

Breeding Habit

The flowers of *Delphinium* are so constructed that outcrossing appears to be the general rule. Each flower has numerous stamens and three separate pistils. Both the filaments and styles are deflexed and not functional in the bud. The stamens mature first; as they mature they become gradually erect, two or three at a time; when erect they open and disperse their pollen, and then bend downward and wither. When all the stamens have shed their pollen the styles become erect, the bifid tips then spread and become receptive. The principal pollinating agents in nature appear to be species of the bumble bee, *Bombus*. The habit of these insects is to visit the lower and older flowers first and then, circling upwards, to visit several younger flowers of the raceme, but not all of them. In this way, flowers with receptive stigmas are visited before the insect encounters pollen of the same plant. It then flies to another plant, sometimes one near at hand, sometimes one farther away. Any given flower is not likely, therefore, to be selfed, nor is it likely that pollen from one flower will frequently be carried to another on the same plant unless a second visit is made. Delphiniums are self-compatible, however, and selfing probably does occur naturally to a certain degree when some stamens lag in anthesis; or if the plant is branched and an insect visits two or more branches. Selfing may also result occasionally from the activities of other pollen vectors, such as hummingbirds, which follow no set pattern in visiting the flowers. Outcrossing appears, therefore, to be the rule, the possibility of selfing not being altogether excluded.

The total seed set varies greatly both within and between species. The mean number of seeds per flower appears to be relatively constant, perhaps about a hundred, but the number of flowers on a given plant and the mean number on different species may vary greatly. The flowers on a vigorous individual of *D. variegatum*, for example, are seldom more than 8; on *D. gypsophilum* they may be as many as a hundred. The number may vary on a given plant from season to season, according to its thrift. If we allow a mean of 10 flowers per individual of any delphinium we have considered (which is low), we might therefore expect a seed set of approximately 1,000 seeds per plant. A colony of 200 plants, which would not be an unusual number flowering in a good year, might scatter a quarter of a million seeds.

The seeds are scattered promptly after maturing but our observations indicate that the great majority probably fall to the ground within a few inches of the female parent when the stalks are shaken by the wind. The follicles are erect and do not usually open completely; hence the seeds of each follicle are scattered gradually. On occasion they may be scattered over a radius of several feet. The dried stalks are elastic and would yield to the pressure a passing animal might make, springing back, they would throw the seeds a short distance. The outer seed coats of some species are loose and more or less inflated, and the seeds of *D. hansenii* are covered with flattened processes. These characters suggest the possibility that, with air turbulence, some

seeds may be caught up by the wind and carried greater distances, or be caught in the coat of an animal. But we have no evidence of this and the morphological differences between closely adjacent colonies suggest that neither pollen nor seeds are carried very far except on rare occasions or, if so, that they are not effective.

Adjacent colonies of *D. parishii,* for example, may differ conspicuously and completely in a morphological character (Lewis and Epling, 1952); all of the plants in one colony may have puberulent stems, and all of those in the next may be glabrous. Variation between colonies of *D. hansenii* is similar. The colonies of this species have dark blue flowers throughout the Kern River drainage, but we know of one colony of at least 50 individuals in this area, all white flowered, which exists within 300 yards of a colony of which all of the flowers are blue. Both colonies are diploid. The hybrid between these two color extremes is known from garden crosses to be of an intermediate color which could be readily observed in either population if it should occur.

Even more convincing of the narrow radius of seed dispersal are the differences in chromosome number between closely adjacent colonies. The colonies graphed in Figure 2 consist of *D. hansenii* or *D. gypsophilum,* or of both. Both may be either diploid or tetraploid (tetraploid *D. gypsophilum* not shown in this figure occurs in the adjacent area). Repeated sampling of the colonies represented shows that, so far as one species or the other is concerned, no mixtures of diploids and tetraploids occur. Tetraploid *D. hansenii* may occur, for example, with diploid *D. gypsophilum* in this area, but we have never found both diploid and tetraploid *D. hansenii* (or *D. gypsophilum*) in the same group. The hybrids between these species, which are referred to elsewhere in this paper, are either interspecific diploids, triploids, or tetraploids. Triploids of the same species in one colony are rare. It is reasonable to suppose that if seed dispersal is fairly wide, both diploids and tetraploids of the same species would sometimes occur together. This does not seem to be true in this area. An alternative explanation, of course, is that the two types have sufficiently different adaptive values as to preclude the seeds of one becoming established in the territory of the other. But this explanation is not convincing in view of the relatively uniform aspect of the sites in this small drainage. We are left with the conviction, confirmed by repeated observation, that a distance of no more than one- or two-tenths of a mile, even without apparent differences of site and associates, forms a strong barrier to effective transfer between colonies of both pollen and seeds.

Genetic interchange between colonies is also apparently limited by a narrow range of conditions for germination and establishment. We have made numerous attempts to establish artificial populations in nature by broadcasting surplus seed lots of which the mean viability was known, and also by transplanting one, two and three year old dormant plants. The genotypes involved were diverse in character and origin, representing not only the wild species but also various hybrids and backcrosses. The seeds were gathered in the garden in early summer and were broadcast in various sites in areas about 10 feet square. Most lots contained 10,000 seeds or more and the total sown was approximately a third of a million, roughly what an average colony might produce in one season. The sites were subsequently inspected each year during the flowering season. Most of the sites chosen were closed communities in which a wild species was actually growing (table I). Two lots were sown in burned areas shortly after burning, one in the Santa Monica Mountains and one near Berkeley. The latter was kindly sown by Prof. Stebbins. No delphiniums were growing in either of these.

TABLE I *Seeds sown in natural environments*

Year	Locality	Approximate number of seeds	Number established
1946	Contra Costa County: east base of Mt. Diablo	1,000	None
1944	Kern County: McKittrick (1 mi. north)	10,000	None
1946	McKittrick (1 mi. north)	20,000	None
1947	McKittrick (1 mi. north)	12,000	None
1944	Bakersfield (4 miles west)	10,000	None
1944	Near Caliente	10,000	None
1946	Near Caliente	20,000	None
1944	Bodfish (3.6 mi. south)	10,000	None
1946	Bodfish (3.6 mi. south)	20,000	None
1944	Walker Pass Summit	10,000	None
1946	Walker Pass Summit	20,000	None
1944	Los Angeles County: Santa Monica Mts. (Mulholland Dr.)	10,000	75 plants
1947	Santa Monica Mts. (Sepulveda Blvd.)	12,000	None
1944	University of California Campus	25,000	None
1946	Madera County: Califa.	3,000	None
1944	San Luis Obispo County: Santa Margarita	10,000	None
1946	Santa Margarita	20,000	None
1947	Santa Margarita	12,000	None
1944	Near Pozo	10,000	
1946	Near Pozo	20,000	One plant
1947	Near Pozo	12,000	
1944	La Panza Grade	10,000	None
1946	La Panza Grade	20.000	None
1947	La Panza Grade	12,000	None
1947	Tulare County: Earlimart (0.6 mi. north)	12,000	None
	Total	331,000	76 plants

Germination is known to have occurred in only three of these sites, the two burns and the closed community near Pozo which is occupied by *D. variegatum*. Germination was abundant in the burn near Berkeley but the seedlings were destroyed by birds, according to Prof. Stebbins. Establishment has taken place thus far only in the two remaining sites. About 75 plants of varied origin have persisted in the burn in the Santa Monica Mountains. One plant has persisted in the site near Pozo.

The latter is of especial interest. About 42,000 seeds were sown at this site from 1944 to 1947. *D. variegatum* appeared most years, but no evidence was found of any other type until 1952 when a single individual of *D. hansenii* was found in full flower, together with the other species. The size of the plant indicated that it was at least two years old. It may have persisted since 1944, forming only rosettes until the present year.

Differences in the requirements for germination and persistence are indicated by the more frequent survival of dormant transplants (table II), but conclusions cannot yet be made as to the degree of establishment because of the possibility that many transplants are still alive but dormant, or are forming only rosettes. The data suggest a gradual decline in numbers.

These experiments indicate that seeds may sometimes germinate and that individuals may sometimes become established far out of their normal ranges, but that numerous trials are probably necessary. They also indicate a greater probability of establishment in disturbed areas rather than in closed communities. They suggest also that new colonies are frequently established from a single source.

TABLE II. *Seedlings and mature plants transplanted to natural sites*

Date	Locality	Material	Results
1947	Contra Costa County: Near Byron	*D. recurvatum* × *hesperium* hybrids 4″ pots	No establishment
1947	Fresno County: Coalinga (10 mi. north)	250 *D. recurvatum* × *hesperium* hybrids 4″ pots	1948 2 rosettes 1949 no plants 1950 no plants 1951 no plants 1952 no plants
1946	Kern County: Bodfish (3.6 mi. south)	Seedlings	No establishment
1947	Walker Pass	148 *D. hansenii* × *parishii* hybrids 4″ pots	1948 7 rosettes 1949 no plants 1950 2 small rosettes 1951 no plants
1950	McKittrick (10 mi. west)	1300 plants from 4″ pots mixed origin	1951 many rosettes 1952 7 rosettes 4 flowered
1946	Los Angeles County: Pearland (2 mi. east)	Seedlings	No establishment (eaten by rodents)
	Pearland (3.5 mi. n.e.)	300 plants from 4″ pots mixed origin	No establishment
1947	Santa Monica Mts. (Sepulveda Blvd.)	1000 plants from 4″ pots mixed origin	1948 25 rosettes 1949 6 fl. (no seed set) 1950 2 fl. (no seed set) 1951 no plants 1952 5 flowered
1946	San Luis Obispo County: Guadalupe (4.8 mi. north)	Seedlings	No establishment
1947	Pozo (1.2 mi. east)	60 *D. parryi* × *variegatum* hybrids from 4″ pots	1948 4 rosettes 1949 1 plant (flowered) 1950 no plants 1951 no plants 1952 no plants

Similar but less extensive experiments with seeds of two species of *Salvia* and four of *Lepechinia* lead to the same conclusion.

In view of these experiences we are therefore inclined to the belief that pollen and seed dispersal of *Delphinium* are generally confined to the immediate vicinity of the parent, that migration of seeds from one colony to another is infrequent or rare, and that the establishment of seedlings is even more rare. We believe accordingly that most colonies are derived from very meager beginnings, perhaps from the establishment of a single seed, and that their variability derives chiefly from this source. Each colony is likely to represent an independent establishment which persists largely by virtue of its own adaptive resources in relation to the environmental rhythm of the site it occupies, being modified by introduction of new genotypes only on rare occasions.

DISCUSSION

The species we have studied occur in a region of low precipitation, where rain falls predominantly during the coldest

months of the year. Vegetative growth occurs during this period, followed by flowering and seeding during the warmer months of spring and early summer. After this, the aerial portions of the plants wither and die and the root and crown become completely dormant for the remainder of the year. This cycle is primarily dependent on the water relation. When the rains cease the ground may dry very rapidly. The shallow roots are affected correspondingly. The cycle can therefore be interrupted at any point by a return to dormancy which is seldom if ever broken until the following year and may persist indefinitely, dependent on the weather.

The intensity and duration of winter storms and the conditions of temperature and wind which follow them, conserving or dissipating the soil moisture and modifying the saturation deficit of the air, are highly variable from place to place and from year to year. The weather of each site is likely to be different and that of a given site is different in successive years. Our observations and experiments indicate that the members of a colony respond differently to these yearly differences of weather. Some may remain dormant, others may grow vegetatively for a while and then become dormant; still others may flower and set seed. Those which produce offspring in response to each successive environment tend to be different individuals and one may therefore suppose that recombination and hence the adaptive mode of each succeeding generation produced by a colony is different. The offspring produced during periods of drought would presumably tend to be better adapted to these conditions. Differences of this sort within colonies would be further secured and maintained by any conditions which would reduce or localize crossing over. Yearly differences of recombination may also be brought about by differences in spacing of individuals. In more mesic sites and during more mesic conditions, the flowering individuals are more numerous and are in relatively close contact. The numbers expected to recombine their genic materials would be greater than in more xeric sites and during years of drought when flowering individuals are few and tend to be more widely spaced.

It would appear, therefore, by virtue of this faculty of a differential response to weather by assuming dormancy or failure to flower, that the breeding group of a colony may be reconstituted from year to year and that a population may be parcelled in time, in a way parallel to the parcelling in space which Wright (1931, 1948) has suggested is the optimum condition for evolution, even though the total membership of a colony remains fairly stable from year to year.

The degree to which a colony would respond differentially would depend on its habitat with reference to the climatic gradient. Bearing in mind the effect of topography, rainfall tends to decrease and mean temperatures to increase from north to south and from the coastal drainages to the interior, as we have observed above. The amplitude of fluctuations also corresponds generally to the gradient, the greatest being found in the interior.

In North America *Delphinium* is chiefly associated with elements of the Arcto-Tertiary Flora. It is generally found in mesic sites. The species now associated with the communities of the Madro-Tertiary Flora are few and appear to be exceptions, particularly in view of the fact that they flower and reproduce only or chiefly during the more mesic aspects of these communities. The trend of evolution seems clearly to have been from the more mesic to the more xeric. The optimum of most species would accordingly be expected in areas of greater and more uniformly distributed rainfall and of more equable temperatures. Sites near the optimum for the species concerned would be subject to less fluctuation than those toward the more arid periphery. The central populations would

tend toward greater adaptive similarity. Recombinations would tend toward greater uniformity and replacement of individuals would be more gradual. Populations in such sites would also be less subject to decimation during environmental extremes and hence would not often be reestablished from limited and perhaps exceptional sources.

The yearly differential within a colony in response to weather would be accentuated by another characteristic of the climates concerned. The fluctuations of weather are not erratic but tend to follow in sequence so as to produce an alternation of wet and dry phases which may last for several or many years. During the decade we have studied this group the weather has followed a trend of increasing drought and we have reason to believe that the individuals which have produced seed during this period have been generally adapted to a relatively xeric habitat in a way that those which have remained dormant are not. One might therefore expect an increase of genotypes during this period which lie at one extreme of the adaptive range of the colonies concerned.

We have already learned that very few seeds become established beyond the confines of the colony in which they are produced. An increase of genotypes adapted to the xeric aspects of a site would accordingly increase the possibility of their establishment in similar sites elsewhere. Given now the faculty of dormancy, the occupation of still more xeric sites would be fostered and the ecological range of the whole species increased.

Consideration of the two colonies of the Marsh Creek Road, previously referred to, will suggest how this may come about. The site near Mt. Diablo is more mesic and comes under the coastal influence. That near Byron is much more xeric and comes under the influence of the diminished rainfall and higher temperatures of the central valley. It still lies within the oak woodland, and the vegetation is predominantly coastal. The environment at Byron during the moist phase of the weather trend is more mesic than that of the drier phase near Mt. Diablo. Let us suppose that some of the seeds produced by the colony near Mt. Diablo during a period of drought were to be carried to a site like that at Byron. This event would not occur during every dry phase of the weather; but it might occur during the long course of time and among these seeds a genotype might be expected which would be adapted to the mesic aspects of this more xeric habitat. This dispersal need not take place at once over this distance, which is roughly 10–12 miles in an airline; it might progress from station to station over shorter distances, for we know of intermediate colonies. Having arrived at this site and having germinated, it could survive there for an indefinite period in a vegetative state, either forming rosettes during winter, or remaining dormant. With the advent of conditions which would permit completion of its reproductive cycle it would scatter seeds. A single plant might reproduce by self fertilization under these conditions because of self-compatibility. Should more than one adapted seed have been transported to the site and more than one seedling established, a limited amount of outcrossing might occur. In either case the colony might gradually increase. With mutation and further recombination, this colony might become in turn a "staging area" for further advance into the periphery.

Should peripheral colonies of *Delphinium* be established by a process such as this, then one would expect that each would be relatively uniform and that inter-colonial differences would be greater along the ecological periphery of a species than towards its optimum. This seems to be true. Comparisons between colonies of characters which are readily determined and scored, such as pubescence and flower color, show that ecologically peripheral colonies have greater inter-colonial differences than exist between colonies closer to the ecological optimum

and that characters which distinguish a marginal colony often occur as sporadic variants in colonies toward the center (Lewis and Epling, in press). This condition might have come about by fragmentation of a once widespread population because of ecological restriction and differentiation, followed by a differential increase of genotypes adapted to each site. Nevertheless, the evolutionary trend of the whole genus, and the limitations on establishment which we have discussed, suggest rather that these differences have come about in the way we have outlined.

Summary

The genus *Delphinium* is holarctic and characteristically occupies relatively mesic habitats. The adaptive character of a species group associated with more xeric conditions has been examined with the purpose of determining the means whereby the adaptive range of the genus has been expanded.

The species concerned are primarily insect pollinated and outcrossed. Seeds and pollen are but seldom carried beyond the limits of the colony, or if so are usually ineffectual. This is indicated by observation and extensive attempts to establish populations in nature from seeds and transplants.

The primary variable in the adaptedness of this group is probably the water relation. The climate concerned is not only arid but is characterized by marked fluctuations in weather, particularly in rainfall, from place to place, from year to year, and at the same time over longer periods during which drought may be severe and sustained.

The adaptive response in this group of species is found in dormancy which can be initiated at any stage of the growth cycle, and once initiated is not broken until the following growing season. By a differential response of the individuals in a population some may remain or become dormant in one season while others complete the growth cycle and are thus a part of the breeding population in that season. By virtue of the differential adaptedness of the genotypes within a population the breeding group is composed of different individuals each year. One may, therefore, suppose that the adaptive mode of each succeeding generation produced by a colony is different. The offspring produced during periods of drought would tend to be adapted to these conditions. An increase of genotypes adapted to the xeric aspects of a given site would be expected as a result of prolonged drought, and would increase the chances of their establishment in more xeric sites elsewhere. It may be by such means that *Delphinium* and other groups have been able to extend their adaptive ranges.

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THE BIOLOGICAL STATUS OF *BETULA ANDREWSII* A. NELS.

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INTRODUCTION

It has been known for many years that interspecific hybridization within the genus *Betula* occurs more or less freely in nature. Woodworth (1929) states that at least 15 recognized hybrids occur in the genus, including *Betula pumila* × *Betula lenta, Betula populifolia* × *Betula papyrifera, Betula verrucosa* × *Betula papyrifera,* and others. Rosendahl (1916) also reported and analyzed hybridization within the genus, including a cross involving *Betula papyrifera* and *Betula pumila* var. *glandulifera.* In addition, *Betula* is known to be a polyploid genus (Woodworth, 1929). This paper deals with the first instance in which hybridization has been reported involving *Betula papyrifera* and *Betula occidentalis.*

Wherever two species likely to hybridize grow together, and conditions are more favorable to one than to the other, introgressive hybridization may occur. Anderson (1949) has described this phenomenon as a contamination of the germplasm of one species by the germplasm of the other. The species less favored by present conditions may gradually be absorbed by the other, and the external characteristics of the hybrid derivatives will be more and more those of the best preadapted species.

In the fall of 1906, Mr. Darwin M. Andrews, a nurseryman, discovered a small grove of paper-barked birches on the northwest slope of Green Mountain, a high foothill immediately southwest of Boulder, Colorado. Dr. Aven Nelson, to whom Andrews had sent specimens of the birch for identification, published a note (Nelson, 1907) drawing attention to the Andrews collection. The somewhat atypical character of the specimens and the geographic isolation of this birch community led Nelson to believe that these plants might constitute a new species, distinct from *Betula papyrifera* Marsh, the common paper-barked tree of northern and eastern North America. However, because of the incompleteness of the specimens before him, and because of his inexperience with the population in the field, Nelson only tentatively named this birch "Betula andrewsii," which name was, of course, a ***nomen provisiorum*** and therefore not valid until its subsequent publication in Coulter and Nelson, *New Manual of Botany of the Central Rocky Mountains* (1909).

Bertram T. Butler (1909), in his taxonomic revision of the paper birches, did not think there were sufficient grounds for separating *Betula andrewsii* from *Betula papyrifera.* Dr. Francis Potter Daniels (1911), in his *Flora of Boulder, Colorado, and Vicinity,* formally reduced *Betula andrewsii* to a trinomial, *Betula papyrifera andrewsii* (A. Nels.) Daniels. Daniels' reduction of *Betula andrewsii* to subspecific rank may have been a factor in a subsequent neglect of the problem. At least, recent authors of tree manuals have not attempted to determine further the possible relationships of *Betula andrewsii.* The status of *Betula andrewsii* has remained unsettled to the present time.

The herbarium material of this taxon consisted, until recently, of the type collection (a branch with yellowed leaves and fragmented aments, plus a strip of bark) collected in the fall of 1906, and a branch collected early the following year. This collection is in the Rocky Mountain Herbarium of the University of Wyoming at Laramie. A photograph of the tree

from which the type specimen was collected was published in Nelson's original article. It is believed that the type collection is all that was available to Nelson at the time of the original publication.

In 1946 the population was "rediscovered" in what is now known as Long Canyon (U.S.G.S., T. 1 S., R. 71 W., Sec. 2, Eldorado Springs, Colorado, quadrangle, 7½ minute series, 1944). By careful comparison, the writer has established beyond reasonable doubt that the Long Canyon population is the same group of trees which were described by Nelson. In fact, the type tree was identified by matching it with the published photograph.

Long Canyon is a steep-walled ravine extending for about three-fourths of a mile in a southwest to northeast direction and emptying into Chicken Ranch Gulch at the head of Gregory Canyon. The altitude at the southwestern, or upper, end of the canyon is approximately 7,250 ft., and the altitude at the northeastern, or lower, end of the canyon is 6,700 ft. It is a well-shaded canyon, much cooler and more moist than the other canyons in the area. The majority of the birches are found within a few feet of the intermittent stream which flows through the canyon.

One of the most interesting features of the canyon is that it contains several rare mesophytic species. The apparent relict nature of some species of the Long Canyon community suggests relationship to similar communities found in the Black Hills of South Dakota. Among the species included in this association are the following: *Aralia nudicaulis, Botrychium virginianum, Carex deweyana, Corylus rostrata, Goodyera decipiens, Lilium philadelphicum, Onoclea sensibilis, Osmorhiza longistylis, Rubus pubescens,* and *Sanicula marylandica. Betula papyrifera* is the most conspicuous woody type.

A casual examination of the area established the fact that *Betula papyrifera* ("andrewsii") was present, along with typical individuals of *Betula occidentalis* Hook. (*Betula fontinalis* Sarg.) which is the common species of birch in Colorado. The entire combined population of birches in this canyon, including both species, consists of 153 trees. *Betula papyrifera* is not found elsewhere in the region. In fact, this is the only colony of paper birch known to occur south of the Black Hills of South Dakota, the nearest locality where the eastern subspecies, *Betula papyrifera* Marsh var. *papyrifera,* occurs; and western Montana, the nearest locality for the western subspecies, *Betula papyrifera* Marsh var. *commutata* (Regel) Fernald.

Despite the fact that it was easy to distinguish some individuals which clearly belonged to either of the species of birch named above, the remainder appeared to be so variable as to make it impossible to categorically assign one or the other species name to them on the basis of characters normally used in identification. This was surprising, because the two species are distinguished by characters which are essentially qualitative and constant. In other words, wide variations of the two species in the direction of each other were noted throughout the colony. This variation pattern was one of the reasons why Nelson was dubious of his identification of Andrews' specimens.

The widespread blending and recombination of species characters among the trees in the Long Canyon colony strongly suggested that natural hybridization might be the cause of the variation pattern. Therefore, a detailed analytical population study was felt to be necessary for an understanding of the biological structure of the population, and the present investigation was begun. The methods used in the following analysis are essentially those devised by Anderson and co-workers in their highly successful studies in introgressive hybridization.

ACKNOWLEDGMENTS

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especially grateful to Dr. W. A. Weber, his graduate advisor, for guidance and criticism in the research and in the preparation of the paper; to Dr. C. L. Porter, of the Rocky Mountain Herbarium of the University of Wyoming, for the loan of type specimens; and to Mr. Harold E. Malde, of the University of Colorado Museum, for the photography.

Methods

The methods employed in the study are as follows: A true compass course of the canyon was laid out and mapped. Each tree in the colony then was numbered, permanently tagged, and its position indicated on the map. Mass collections, as described by Anderson (1941), were made in the following manner. Several branches were taken from each tree in the colony. These selections were made from corresponding positions on each tree so as to minimize the possibility of error resulting from environmental modifications. Each sample included a number of mature leaves, a twig of the current year's growth and the previous year's growth, and, whenever possible, a sample of the fruit. The series of collections was made during the latter part of August and the first part of September, 1949. The complete collection (153 sheets), and all documentary materials, are preserved in the University of Colorado Herbarium. Notes were also taken for each tree concerning the size of the largest shoot (diameter breast high), number of shoots per tree, height of the tree, color of the bark, exfoliation, flowering time, and the leaf-maturity time.

Each character was analyzed in turn, and graphs were constructed to show correlations and frequencies. A system for a comparative study known as the polygonal graph method, described by Davidson (1947), was employed. Basically the graph consists of a circle, with as many radii as there are characters to be compared. The characters, measured along each radius, are assigned absolute, relative, or arbitrary values. The character values exhibited by each specimen are plotted individually along each radius, and the points so plotted are joined. Thus, each specimen is represented by a polygon, and the entire population sample is represented by a series of such polygons. The advantage of polygonal graphing is that members of a population may be compared with respect to a number of varying characters at a glance (see fig. 1).

The final method of analysis employed in the study was the hybrid index method, described by Anderson (1949). To each characteristic an index number is assigned, which is always 0 for the condition typical of one of the parental species, and may vary from 1 to 4, depending on the character, for the condition typical of the other. The characters are weighted according to their importance as diagnostic characteristics of the species concerned. Greater weight is attached to characters which are known to be relatively constant in regions where each of the parental species occurs by itself. After the index numbers for each of the

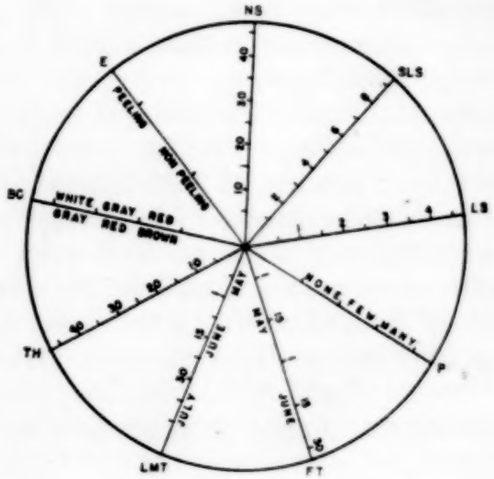

Fig. 1. Form used in the construction of the polygonal graphs, showing the basis for graphing of nine characters. The symbols used are as follows: NS, Number of shoots. SLS, Size of largest shoot—d.b.h. in inches. LS, Average maximum leaf size in square inches. P, Pubescence of twigs. FT, Flowering time. LMT, Leaf maturity time. TH, Tree height in feet. BC, Bark color. E, Exfoliation.

characters have been determined for each sample studied, the numbers are totaled. This total is called the "hybrid index value" of the sample. A bar graph is then constructed on which the total index values are plotted against their frequency within the population. Thus, the probable presence or absence of hybridization between the parental forms can readily be determined by a study of the peaks occurring on the graph.

Betula occidentalis, or river birch, is the most common birch of western United States and Canada. It is found along streamsides in mountainous areas from British Columbia to New Mexico and Arizona. It is a tall shrub from 10 to 20 feet in height, having many more or less equal stems, each usually not exceeding 4 inches in diameter; the bark is thin, non-exfoliating, shiny and dark reddish-brown, remaining the same color in mature trees; the lenticels are usually elliptic to oblong; the twigs exhibit no pubescence but are glandular-warty; the leaves are small (¾ to 2 inches long), broadest near the middle, acute at the tip, dark green and glabrous, with a sharply double-serrate margin.

Betula papyrifera, the paper or canoe birch, is one of the common forest trees ranging from eastern U. S. northwestward throughout Canada. It is a medium-sized tree rarely over 80 feet in height or 2 feet in diameter, having a single stem or a maximum of a few stout main stems, each seldom less than 8 inches in diameter at maturity; the bark is thin and reddish-brown in the very young trunks, but soon exfoliates and exposes successive underlying layers of the chalky-white, papery bark which is typical of the mature trees; the lenticels are elongate and linear; the twigs are pubescent and glandular; the leaves are medium-sized to large (1½ to 4½ inches long), broadest near the base, strongly acuminate at the tip, dark green and glabrous above, pubescent beneath, with an irregularly- or doubly-serrate margin. This species has many varieties and forms, and is a particularly complex and poorly understood group, especially in the northwest. Dr. Marion Ownbey of the State College of Washington, in correspondence, mentions that the taxonomy of the forms of paper birch in the northwest (where the ranges of the two species described here overlap) is especially difficult because of intergradation between various races and species of birch in the region.

In most of the studies concerning introgressive hybridization among the angiosperms, for example, those on *Iris* (Anderson, 1936a), *Helianthus* (Heiser, 1947), and *Tradescantia* (Anderson, 1936b), three distinct groups of plants have been available, namely, relatively pure populations of each of the two parental species, plus a hybrid zone or swarm composed of the segregating offspring of the cross. The presence of pure segments of the parental species is important because the variation patterns of these parents may be used as yardsticks for comparison with the variation pattern of the hybrid swarm. Under such conditions it is possible to determine the relative success or failure of the F_2, or of back-crosses of the F_1 to either parent. In addition, it is also possible to study the ecological niches occupied by the parents and to find reasons why the barriers which ordinarily separate the species may have broken down.

With these considerations in mind, there were certain difficulties inherent in the Long Canyon birch problem. *Betula papyrifera,* as it occurs in this area, is a decimated relict population, consisting of only a few recognizable individuals. In order to find a pure population of a large enough size for statistical equivalence to pure populations of *Betula occidentalis* in this region, one must go to the Black Hills, the nearest place where paper birch occurs in pure stands. It would be precarious to rely on such a population as representative of the parental stock of the Long Canyon birches, since there is no assurance that the popu-

lation genotype of the Black Hills colony is the same as that of the Long Canyon remnant.

Nevertheless, the writer made mass collections of pure populations of each parental species, including Black Hills material of *Betula papyrifera,* for comparative study. A population sample of pure *Betula occidentalis* (W. A. Weber, No. 5945) was obtained in Boulder County, Colorado, along the banks of Middle St. Vrain Creek between Peaceful Valley and Raymond, 8,200 ft. alt., Aug. 21, 1950. A similar sample of *Betula papyrifera* (W. A. Weber, No. 5946) was obtained in Custer County, South Dakota, in the second draw west of Legion Lake, along Galena Creek, 4,500 ft. alt., T. 3 S., R. 5 E.; Custer State Park, Aug. 30, 1950.

Because it was impossible to divide the Long Canyon birches into two clear-cut groups, the colony was analyzed as if it were a single variable population. This was done despite the obvious fact that the population had at its extreme limits of variation two very distinct taxa which are universally accorded specific rank by competent taxonomists. The aim of this work was to establish whether the intergradation observed was real or apparent, and if it were real, whether the cause of the variability might be attributed to hybridization. It should be pointed out here that the evidence presented in favor of the latter hypothesis thus far is indirect and circumstantial, inasmuch as the proof lies in more intensive studies of the cytology and genetics of the two species.

Analysis of the Variation Pattern

The stem or shoot system

The great majority of the trees in the population appear to be mature, for reproduction is very slow, as is attested by a lack of seedlings or saplings in the area. For this reason, error introduced by the presence of several age groups is believed to be negligible. As the stems attain a certain size or age they die, decay, and eventually fall, and are replaced by the younger shoots. Thus, the age of one individual stem is no indication of the age of the tree. Borings were made on several of the larger trees, including the type tree of *Betula papyrifera andrewsii,* and the age of the oldest shoot was determined to be 89 years, but the tree itself may be presumed to be much older.

A scatter diagram was constructed of the entire population, plotting the number of shoots per tree against the size of the largest shoot (fig. 2). This was an attempt to determine whether or not a correlation existed between numbers and sizes of stems and to detect any well-defined classes. In general, trees with large numbers of shoots were those in which the largest shoot had a diameter under 3 inches (the *Betula occidentalis* type); trees with smaller numbers of shoots were those which had a diameter of from 3 to 8 inches. However, there was no hiatus separating these classes on this basis. On the contrary, gradual intergradation of types was quite evident. In other words, the second class contained *Betula papyrifera*-type trees and

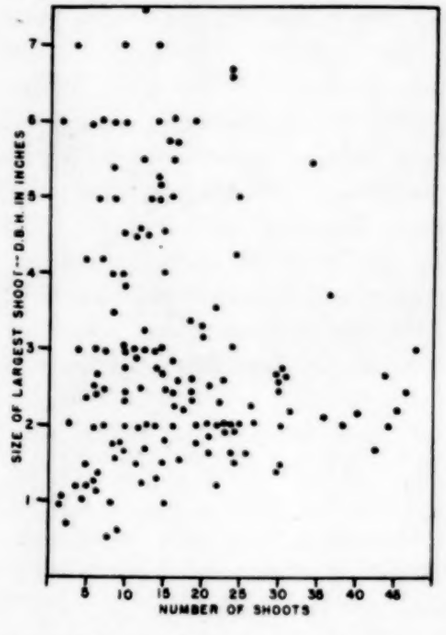

Fig. 2. Relationship between shoot number and shoot size (explanation in text).

intermediates connecting these extreme phenotypes. There were no trees which combined the attributes, large stems and many stems. Such a combination may be physically impossible to achieve. To summarize, there is a very real intergradation in the size and habit characteristics of the stem which are ordinarily used to distinguish *Betula occidentalis* and *Betula papyrifera,* and this intergradation is the result of factors other than developmental ones.

Tree height

Height estimates were made on each tree to determine whether or not any significant group differences were present. In populations having many age groups, tree height might not be a good indicator of specific differences, because the saplings of each species would fall into the same height classes. However, in this colony very little reproduction is taking place, with the result that the colony consists for the most part of mature trees, and therefore, the height estimates may be of some significance. Height varied from 8 ft. to 40 ft. Two principal classes were distinguished. One hundred and fifteen trees were in the 0 to 20 ft. class, and thirty-eight trees were in the 25 to 40 ft. class. The second class thus contains trees exceeding the maximum height found in *Betula occidentalis* in pure stands. This class resembles *Betula papyrifera* as to this character (see also discussion on bark characters). Tree height was the only character which did not show considerable intergradation between types.

Bark characters

Betula occidentalis and *Betula papyrifera* differ by supposedly absolute qualitative character combinations of the bark; namely, dark-colored (red-brown) non-peeling bark, and light colored (white-gray) peeling bark, respectively, on the mature tree. Nevertheless, a great deal of variation both in color pattern and exfoliation was found in the Long Canyon colony. In general, trees with smaller numbers of shoots and larger diameter, breast high (d.b.h.) measurements were of the light, peeling type. No trees with white exfoliating bark had a minimum d.b.h. of less than 4 inches. Smaller trees with larger numbers of shoots were of the dark, non-peeling type. Variation with respect to these characters chiefly was concentrated in trees larger than 4 inches, d.b.h. This variation manifested itself as relative differences in exfoliation and in bark color.

If these characters were simply inherited and not linked, one might expect Mendelian recombination to account for four general classes, namely, (1) plants with dark, peeling bark; (2) plants with light, peeling bark; (3) plants with dark, non-peeling bark; and (4) plants with light, non-peeling bark. Classes 2 and 3 are found as representing the two parental species, and class 1 contains all the intermediate types. 14% of the population falls in class 1; 23% in class 2; and 63% in class 3. Class 4 is, for some reason or other, an unrealized combination. Since even within class 1 there was recognizable variation, it may be assumed that inheritance of bark characters is complex.

Leaves

Betula occidentalis and *Betula papyrifera* differ markedly in the shape and size of their leaves. *Betula occidentalis* has a small leaf, ¾ to 2 inches long, rhombic to ovoid in shape and with an acute tip, whereas *Betula papyrifera* has a medium-sized to large leaf 1½ to 4½ inches long, with an ovate to oblong shape and strongly acuminate tip. Leaf shape and size in the Long Canyon birches were relatively constant on a given tree, but variation between trees was so continuous that definite size and shape classes could not be distinguished (see fig. 3).

The ten largest leaves from the collection of each tree were selected and the marginal outlines were traced on

paper. The minute teeth of the margins were disregarded except where they were so deep as to constitute lobing. A planimeter reading was then made of each leaf tracing and an average was computed, of the ten leaves, for each tree. This measurement gave the average maximum leaf area in square inches. The average maximum leaf size was selected rather than a mean value, because the developmental aspects are thus discounted, and errors in selecting an average size leaf for a given tree are minimized. Thus, the problem concerned itself more in how large the leaves can become rather than with a mean size. Attempts were made to compare the rhombic with the ovate and intermediate leaf shapes, but no adequate method could be found statistically to represent the variation. A bar graph was constructed plotting the frequency distributions of the average maximum leaf areas in increments of $\frac{1}{4}$ inch (fig. 4). The total range of variation was from $\frac{3}{4}$ to $4\frac{1}{4}$ square inches.

The intermediate nature of the Long Canyon population as to leaf size is apparent in figure 4 in which a comparison of leaf size frequency is made involving the Long Canyon population and the pure species from St. Vrain Creek, Colo-

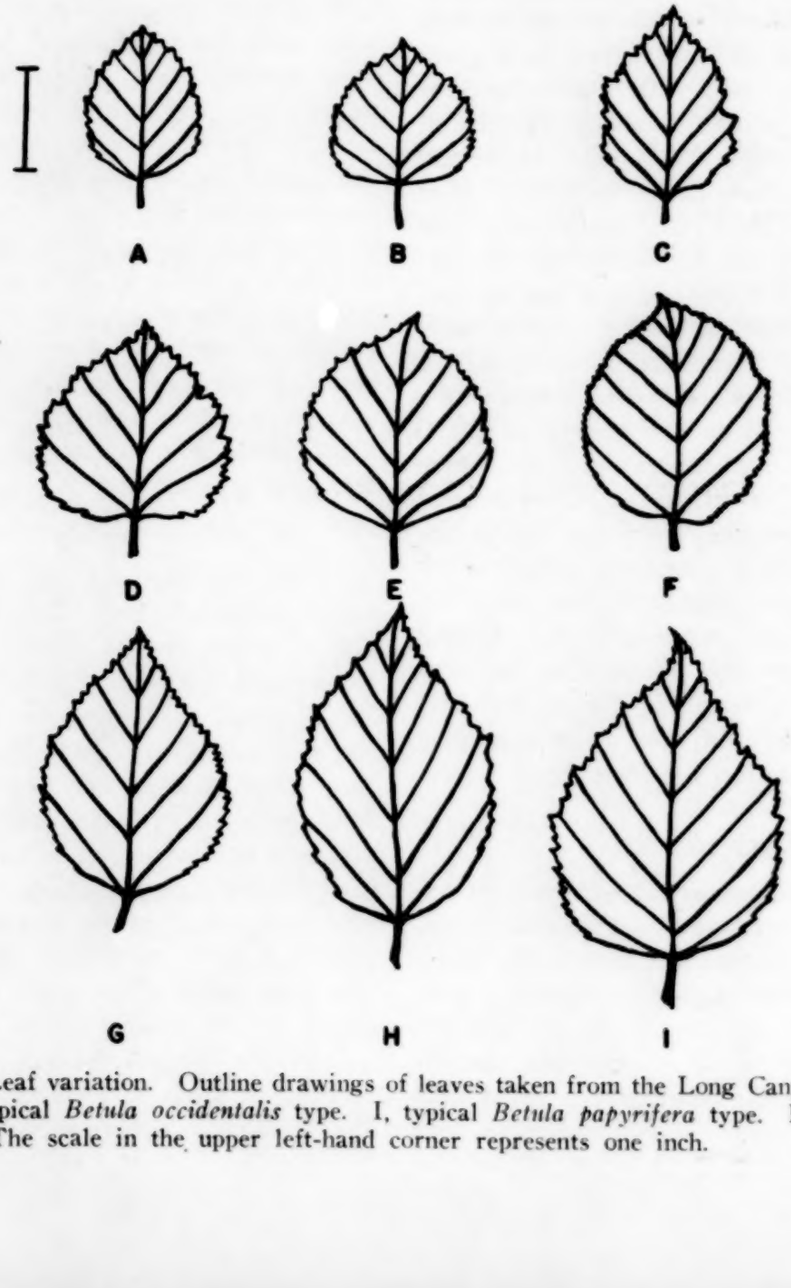

FIG. 3. Leaf variation. Outline drawings of leaves taken from the Long Canyon population. A, typical *Betula occidentalis* type. I, typical *Betula papyrifera* type. B–H, intermediates. The scale in the upper left-hand corner represents one inch.

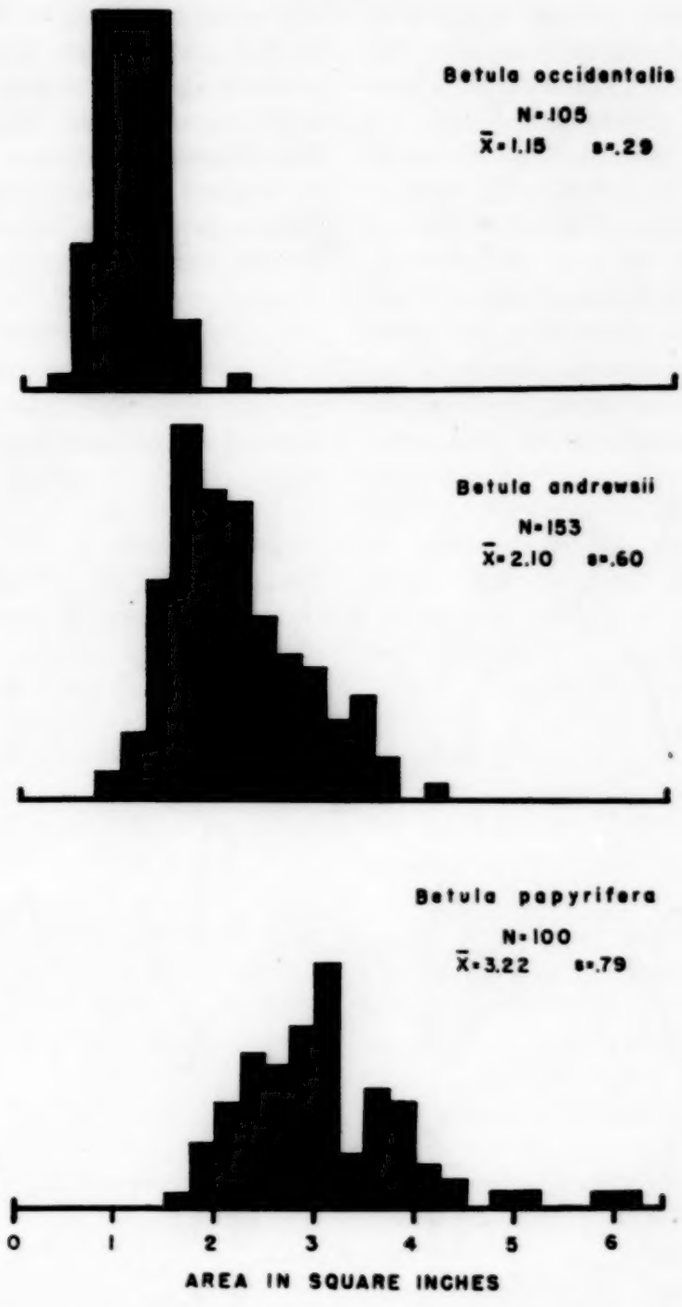

FIG. 4. Average maximum leaf area in three populations (explanation in text).

rado, and the Black Hills of South Dakota.

Pubescence

Betula occidentalis differs consistently from *Betula papyrifera* in its total lack of pubescence. *Betula papyrifera* shows developmental variation involving gradual loss of pubescence with increasing age. The young shoots of *Betula papyrifera*, however, almost always exhibit a moderate to heavy pubescence. In both species this character is only measurable prior to the sloughing of the epidermis, which occurs during the second or third year, at which time the pubescence is lost. Both species have glandular-warty twigs in about the same degree. The leaves of the two species exhibit similar pubescence differences, the leaves of *Betula occidentalis* being totally glabrous, and those of *Betula papyrifera* being pubescent beneath, most noticeably in the vein axils.

Pubescence of the young twigs was chosen as the most useful character for study of pubescence variation. Three classes could be distinguished on the basis of relative abundance of hairs, namely, (1) heavy pubescence (many hairs), (2) light pubescence (few hairs), and (3) complete lack of pubescence. Nineteen trees were found to fall in Class one, eighty-four in Class two, and fifty in Class three. Because the intermediate pubescence class went along with plants which possessed a majority of characters belonging to *Betula occidentalis*, the variation in pubescence could hardly be attributed to the intraspecific range of variation of *Betula papyrifera*. Rather, this phenomenon strongly suggested an infiltration of the pubescence genes of *Betula papyrifera* into the population of *Betula occidentalis*.

Bracts

The floral bracts of the carpellate aments in birches are usually of a distinctive shape, and thus they have been

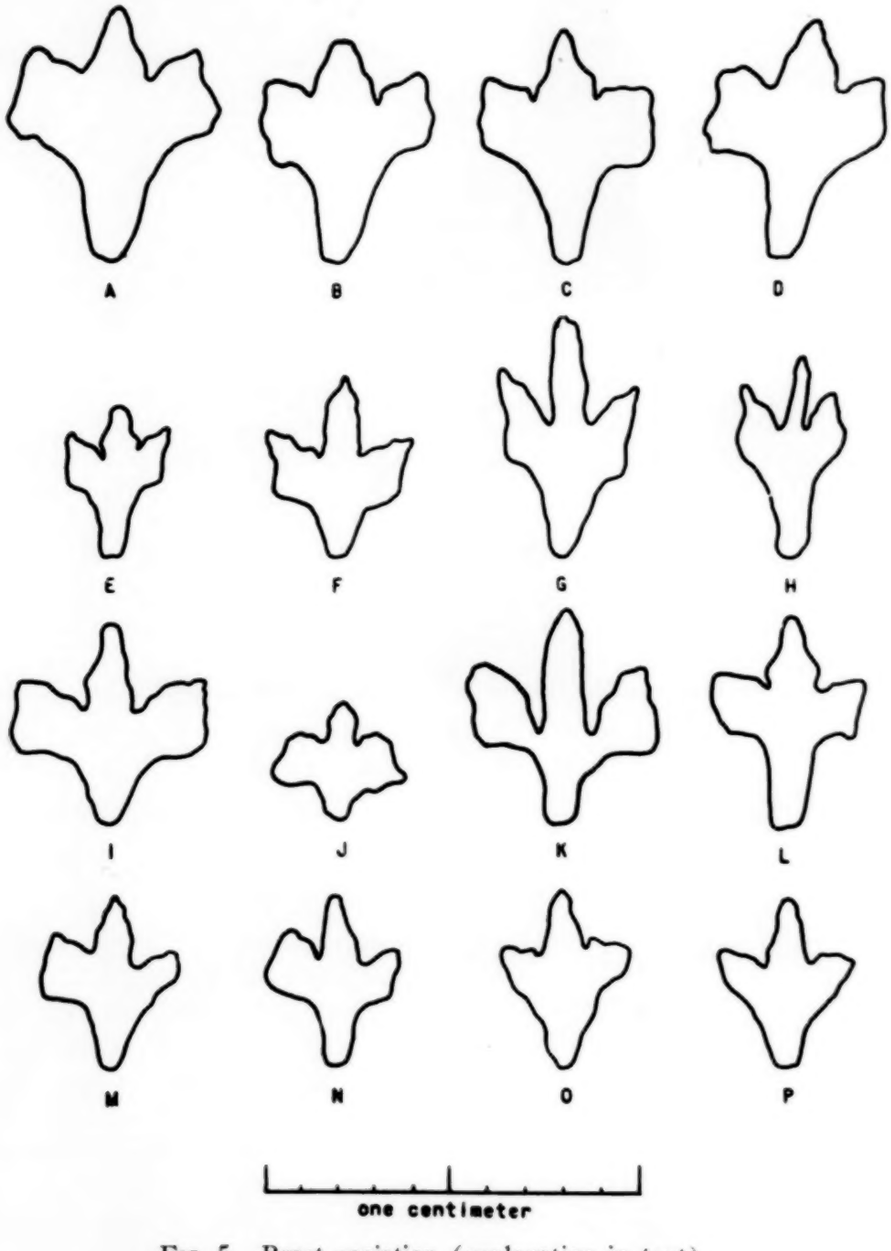

FIG. 5. Bract variation (explanation in text).

used to advantage in distinguishing species. Unfortunately, the bract differences between *Betula papyrifera* and *Betula occidentalis* have never been elaborated in the existing literature. For this reason it is difficult, at present, to evaluate the bract variation in the population under discussion. The outline drawings in figure 5 picture representative bracts of *Betula occidentalis* from a pure population in the St. Vrain Canyon, near Raymond, Colorado (A, B, C, D); bracts from a pure stand of *Betula papyrifera* from the Black Hills of South Dakota (M, N, O), and one bract of the same species from Essex County, N. Y. (P). The two middle rows (E through L) picture a series of bracts illustrating the recognizable variation classes found in the Long Canyon colony. Among the eight classes in the colony, 15% of the plants fell in Class E; 21% in F; 15% in G; 15% in H; 8% in I; 8% in J; 13% in K; and 5% in L. Only 48 of the 153 individuals in the population bore aments in 1949.

Until adequate studies are made on the normal intraspecific variation of the bracts in each parental species, it probably is not safe to speculate on the meaning of the variation found in the Long Canyon birches. Nevertheless, the wide range of variation of the bracts shows a direct correlation with the variation pattern in the other characters studied.

Phenology

In birches, the aments, along with the pollen grains, are formed during the summer and fall previous to their flowering season. Thus, anthesis in birches involves elongation of the aments and discharge of the pollen. In both species anthesis precedes the unfolding of the leaves by almost a month, the former occurring in the middle of May.

It was desirable to determine whether or not the parental types were characterized by a sharp discontinuity in anthesis, since this would constitute a seasonal isolating barrier. A phenological survey was made in the spring of 1950. The area was visited at intervals of one week and careful records were kept of the date at which individual trees flowered and "leaved out."

All of the trees in the population flowered within a one week period, May 17–24. The date of leaf unfolding (this record was based on the date when completely flattened leaves were dominant on the tree) covered a longer period, extending throughout the month of June. Flowering and leafing obviously were complex phenomena, depending on exposure, altitude, location, moisture, and isolation, as well as upon the genetically determined time of these events. Continued observation over a period of years will be necessary before any significant differences may be detected. The march of events in the spring of 1950 appeared to be telescoped by a combination of a very late spring and dry winter, followed by an abrupt transition to summer weather conditions.

The polygonal graphs

The variability of each of the characters made it desirable to devise a simple method by which the total phenotypes of the individual trees could be compared at a glance. Polygonal graphs (as described above) were constructed for each tree in the colony. The nine characters discussed above were used in the construction of the graphs, including the number of shoots, d.b.h. of the largest shoot, the average maximum leaf size, the pubescence of the previous year's twig, the flowering time, the leaf-maturity time, the height of the tree, the color of the bark, and the character of exfoliation. The assigned values for the characters on each of the nine radii of the graphs are shown in figure 1. Obviously all of the graphs could not be reproduced here. The complete set is filed with the collection of the Long Canyon birches in the herbarium of the University of Colorado. However, a typical polygon of each of the parental species

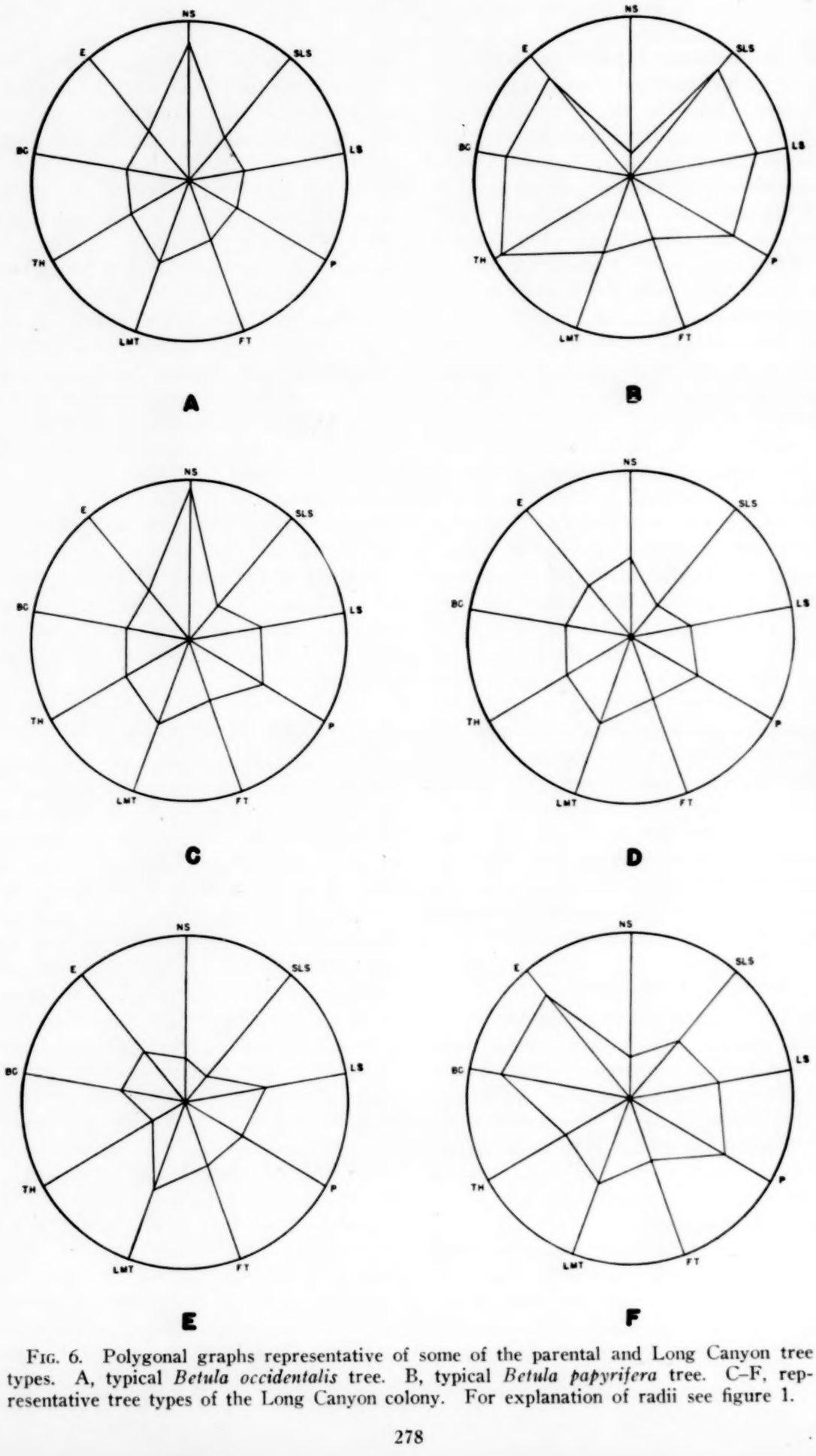

FIG. 6. Polygonal graphs representative of some of the parental and Long Canyon tree types. A, typical *Betula occidentalis* tree. B, typical *Betula papyrifera* tree. C–F, representative tree types of the Long Canyon colony. For explanation of radii see figure 1.

has been included (fig. 6, A and B) for comparison, and to show the marked differences in character. A few selected polygons are also included to show a sample of the variation found in the population (fig. 6, C, D, E, and F).

The polygons were constructed in the hope that the colony could be broken down into groups of trees displaying various recombinations of parental characters. Because of the continuous nature of some of the variation, much difficulty was encountered in trying to assign individual trees to a particular class. However, a rough analysis was made, which is presented below.

Although nine groups of characters were used in making the polygons, only six of these characters are used in the following analysis, namely, those which showed considerable variation and which at the same time were diagnostic features of the parent species. In analyzing the graphs, the writer attempted to demonstrate the number of ways in which the parental characters are combined throughout the population. Because of the continuous variation, it was necessary to make an arbitrary division into two classes for each of the characters. For example, all leaves within the maximum size range of *Betula occidentalis* as it is found in this region in pure stands were placed in the first class, and any leaves with sizes outside the maximum range of this parent were placed in the second class. In other words, the second class contained trees with intermediate-sized leaves in addition to trees having leaves within the normal range of pure *Betula papyrifera*. A similar breakdown was made for each of the characters.

In table I, these characters are referred to by the symbol E for exfoliation, B for bark color, L for leaf size, P for pubescence, S for shoot size, and T for tree height. Capital letters indicate the presence of the *Betula papyrifera* character and lower case letters indicate the presence of the *Betula occidentalis* counterpart. For example, the anagram e b l P s T signifies a tree with non-exfoliating bark, dark colored bark, small leaves, pubescence on the twigs, small shoots, and a tall growth form.

TABLE I. *Combinations of parental characters (explanation in text)*

Combination	Frequency	Combination	Frequency
E B L P S T	22	e B L P S T	0
E B L P S t	4	e B L P S t	0
E B L P s T	1	e B L P s T	0
E B L P s t	11	e B L P s t	0
E B L p S T	1	e B L p S T	0
E B L p S t	1	e B L p S t	0
E B L p s T	0	e B L p s T	0
E B L p s t	1	e B L p s t	0
E B l P S T	4	e B l P S T	0
E B l P S t	0	e B l P S t	0
E B l P s T	2	e B l P s T	0
E B l P s t	3	e B l P s t	0
E B l p S T	0	e B l p S T	0
E B l p S t	0	e B l p S t	0
E B l p s T	0	e B l p s T	0
E B l p s t	2	e B l p s t	0
E b L P S T	0	e b L P S T	0
E b L P S t	0	e b L P S t	0
E b L P s T	0	e b L P s T	3
E b L P s t	0	e b L P s t	12
E b L p S T	0	e b L p S T	0
E b L p S t	0	e b L p S t	0
E b L p s T	0	e b L p s T	2
E b L p s t	0	e b L p s t	2
E b l P S T	0	e b l P S T	0
E b l P S t	0	e b l P S t	0
E b l P s T	0	e b l P s T	1
E b l P s t	0	e b l P s t	43
E b l p S T	0	e b l p S T	0
E b l p S t	0	e b l p S t	0
E b l p s T	0	e b l p s T	0
E b l p s t	0	e b l p s t	38

It should be pointed out that the greatest value in a chart of this kind is that it shows which combinations are actually realized and which are absent. It cannot attempt to explain why certain combinations are absent, but it points up problems arising from the fact that they are absent. Thus, with free recombination of all the characters, 64 classes would be possible, provided a number of conditions were met, namely, (1) that the characters are simply inherited, (2) that they recombine in a Mendelian fashion, (3) that they are not affected

by linkage, (4) that no combination places the plant at a selective disadvantage. Undoubtedly many of the possible combinations are absent because one or more of the above conditions is not realized. Further understanding of the situation lies in the realm of population genetics. It is needless to say that combinations which are not present might be present if the population were larger.

A few general observations may be made concerning the chart. Thirty-two of the possible classes are absent because it is apparently impossible to have plants with peeling bark and dark color, or plants with non-peeling bark and light color. That these factors are developmentally correlated is suggested by the fact that the youngest stems of *Betula papyrifera*-like trees have a dark, non-peeling bark. The combinations which contain the greatest frequencies of individuals are those which combine a majority of the characteristics of one species with an apparently selectively neutral character of the other. For example, the class e b l P s t, with 43 individuals, is essentially a *Betula occidentalis* individual which displays merely the pubescence character of *Betula papyrifera*. Likewise, the class e b L P s t, with 12 individuals, is essentially a *Betula occidentalis* individual which exhibits the pubescence of *Betula papyrifera* and a slightly larger leaf size than is normal for *Betula occidentalis*. Class E B L P s t, with 11 individuals, is essentially *Betula papyrifera* in all its characteristics except in the smaller shoot size and the low growth form. It should be pointed out here, however, that the class E B L P S T (22 individuals) does not necessarily represent pure *Betula papyrifera*, but includes all those intermediate individuals which are outside the normal range of *Betula occidentalis* as to each character. Actually, every *Betula papyrifera*-type tree in the population shows some deviation from the "typical" aspect of *Betula papyrifera* although these deviations would not be apparent to the casual observer.

The hybrid index

A final method, the hybrid index (described above), was employed in order to corroborate the findings of the polygonal graph method.

The hybrid index values were assigned as indicated in table II.

In each character, the index value of 0 is typical of *Betula occidentalis*, and the index value of 4 is typical of *Betula papyrifera*. Variation from the parental forms would be indicated by the intermediate values. A typical *Betula occidentalis* index would range from 0 to 5, whereas a *Betula papyrifera* index would range from 21 to 24.

The total index value for each tree was determined from the polygonal graphs, and a bar graph was constructed

TABLE II. *Hybrid index values (explanation in text)*

Character	Range	Index number
Number of shoots	40–50	0
	30–40	1
	20–30	2
	10–20	3
	0–10	4
Diameter of largest shoot, in inches	0–2	0
	2–4	1
	4–6	2
	6–8	3
	8–10	4
Average maximum leaf size (inches)	.5–1.5	0
	1.5–2.5	1
	2.5–3.5	2
	3.5–4.5	3
	4.5–over	4
Pubescence	no hairs	0
	few hairs	2
	many hairs	4
Dark color	red-brown	0
	gray-red	2
	white-gray	4
Exfoliation	non-peeling	0
	peeling	4

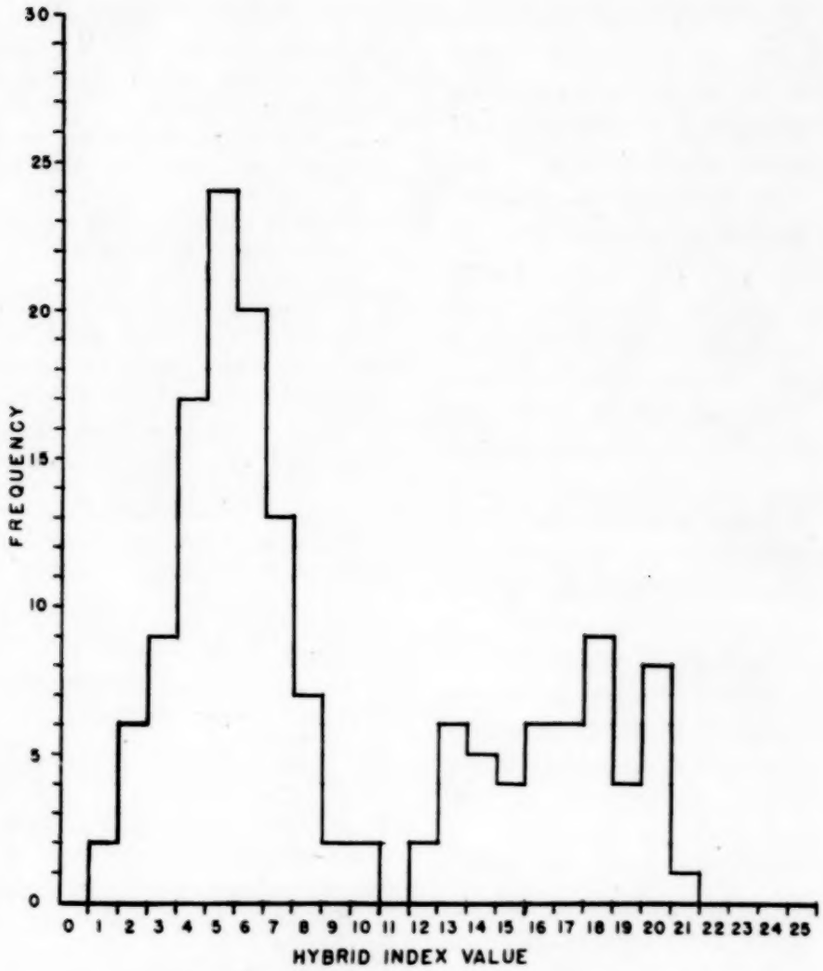

FIG. 7. Hybrid index values of the Long Canyon birches.

upon which the hybrid index values were plotted against their frequencies (fig. 7). It can be seen readily that two peaks are present, one leaning toward *Betula occidentalis* but clearly extending beyond the limiting index value of 5 for this parental form, and the other leaning toward *Betula papyrifera* but having only one tree within the range of what is considered typical for this parent. There is a distinct decrease of frequencies between the index values of 8 and 13. The hiatus between these peaks indicates that absolute numerical intermediates are very rare, the majority of the individuals being those which, as is reflected by their index numbers, resemble to a greater extent one or the other of the parents. The greater frequency of the *Betula occidentalis*-like hybrids is undoubtedly due to the fact that back-crosses would naturally be made to the more abundant parent. Also, strongly intermediate individuals for various reasons may be at a selective disadvantage compared to the parents.

CONCLUSIONS

The evidence presented in the foregoing paper strongly supports the hypothesis that *Betula andrewsii* A. Nels. is a hybrid swarm originating from natural hybridization between *Betula occidentalis* Hook. and *Betula papyrifera* Marsh.

This evidence further suggests that a re-evaluation of the taxonomic problem of the birch species in the northwest may prove successful, if hybridization is taken into account as a possible cause of the complexity of the group.

Summary

1. A colony of paper-barked birch trees, *Betula andrewsii* A. Nels., located in Long Canyon on the northwest slope of Green Mountain, southwest of Boulder, Colorado, was analyzed in order to determine its biological status.

2. *Betula andrewsii* possesses characteristics which indicate that this entity may represent hybrid individuals of a cross involving *Betula occidentalis* Hook., the common birch of the region, and *Betula papyrifera* Marsh, a relict species in this area.

3. Mass collections and detailed notes for analytical studies were made in the summer and fall of 1949 and the spring of 1950.

4. The polygonal graph and hybrid index methods were employed in the analysis, in addition to standard graphing methods, for comparison and correlation of the taxonomic characters of the birches.

5. These studies revealed a considerable amount of recombination of the characters of the two supposed parental species.

6. The results support the hypothesis that *Betula andrewsii* consists of a hybrid swarm involving *Betula occidentalis* and *Betula papyrifera*.

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SPECIATION IN THE FIELD CRICKET [1]

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INTRODUCTION

The common American field crickets have had the generic names *Acheta, Gryllus* and *Gryllulus*. The name now accepted is *Acheta*. The reasons for these changes of name are explained by Gurney (1951). But by whatever scientific name we call the crickets, they are still the common field crickets and very easily recognized as a genus. Splitting the genus into species, however, has been one of the greatest stumbling blocks to taxonomists working on the Orthoptera.

From 1775 to 1903, forty-seven species were described from North and South America and the West Indies. Seventeen names were applied to species occurring within the United States. The diagnostic characters used to separate the species were coloration, size, wing venation and length of such parts as tegmina, wings and ovipositor. All of these characters are extremely variable. As this fact came to be realized a number of the species names were reduced to synonyms. Lutz (1908) published a biometrical study of some large series of specimens from several parts of the United States. He used the length of body, hind femora, ovipositor, tegmina and wings. He found that, with respect to any of these measurements, all specimens fell into one continuous series, except that for length of tegmina and wings they exhibited dimorphism. The species as they had been described and defined by these characters were shown to be mere arbitrary areas in a wide field of variation. Lutz also studied the collection of the British Museum which included specimens of the genus from many parts of the world. These extended the limits of variation of the characters studied but left no gaps. Lutz concluded that no specific entities exist in the genus *Gryllus*.

Rehn and Hebard (1915), who were experienced taxonomists, published a study of the genus seven years later and pointed out that Lutz overlooked certain characters of real specific value in the Old World species of the genus, but agreed with his conclusions as applied to the New World. Rehn and Hebard studied 1,504 specimens from the Western Hemisphere and devised a system of classes for 5 different variable characters. Each specimen was given a set of 5 symbols which roughly described what class of variant it was. While they found that certain variants are more common than others in any geographical region, they concluded that no variant or group of similar variants is sufficiently differentiated or constant to be considered even a geographical race. They reduced all names of indigenous American species to synonyms under the oldest valid name. Some orthopterists continued to use the old names as subspecific names but most of them have now come around to the idea of a single American species. This work greatly simplified the task of identification of field crickets by making it correct to apply the binomial *Gryllus assimilis* Fab. to every specimen of the genus, from Canada to Patagonia.

The present, study, however, shows that this is an over-simplification. It can be shown that even within the limits of the state of North Carolina, there is not just one, but at least four populations of field crickets [2] which may be so reproduc-

[1] Published with the approval of the Director as Paper 408 of the Journal Series.

[2] The approved common name of *A. assimilis* is "field cricket." In this paper the word "field" is usually omitted. It should be under-

tively isolated from each other as to constitute biological, physiological or ecological species, subspecies or races, whichever you prefer to call them.

Evidence for the Existence of Physiological Races

It has been known for a long time that field crickets in the northern states are present in the adult stage over a long period, starting in May and ending with the autumn frosts. It has also been known that crickets which mature in spring survive the winter as nymphs while those maturing in late summer pass the winter in the egg stage. Thus there are two broods of crickets which are prevented from interbreeding by the difference in time of maturity, although there may be a short period in midsummer when adults of both broods are present in small numbers. This isolation could be broken if the spring brood had either a partial or complete second generation, but there is no evidence for the existence of two generations of field crickets in the northern states. Severin (1935), who studied the life history of the field cricket in South Dakota, states that both broods have one generation per year and that they may represent two biological races. The spring brood probably contains less than 5 per cent of the total number of crickets.

Blatchley (1920) retained trinomials for several common variants, including two which he claimed were spring brood crickets in Indiana, namely *Gryllus assimilis pennsylvanicus* Burm. and *G. assimilis vernalis* Blatchley.

Cantrall (1943) analyzed his collections from the George Reserve, Michigan, by classing all specimens according to the symbols devised by Rehn and Hebard. He found that two variants, AVaw3, AVbw3, were the most commonly represented in both spring and fall populations, with 55% of the spring and 94% of the fall brood falling into these two classes. A wider range of variation was found among the spring brood specimens.

Cantrall studied the behavior of the crickets in the field and claims that spring crickets are more solitary in behavior, males being very aggressive, and apt to fight when two of them meet. The fall crickets are more numerous and more gregarious, as many as 26 having been found under a small pile of leaves. No difference in the songs of the two populations was observed. On June 9, 1939, Cantrall placed seven pairs of the spring brood in an outdoor cage. Innumerable young were reared but not one reached maturity by fall and on December 16 only half grown hibernating nymphs could be found in the cage.

On the question of whether the spring and fall broods constitute distinct biological races, Cantrall say that this is a conclusion which may be justified but with which he is not yet ready to concur without reservation. He thinks that possibly there is sufficient overlapping of the two broods to insure intermingling. Differences in behavior may be due to the differences in density of population and other environmental effects.

In North Carolina the first evidence for the existence of a separate race of field crickets came from a distinct type of song, which the writer had never heard in the northern states. This song is a continuous trill very much like that of the 4-spotted tree cricket, *Oecanthus nigricornis quadripunctatus*, but with certain distinguishing characteristics. It is heard in old fields, pastures, lawns, crop lands and roadsides. In North Carolina this song is most noticeable on warm nights in March or early April when the field crickets are the only insects singing. The song is often heard during the day also. Except for a short period in late June, the trilling crickets continue to sing until September. The trilling song was first described by Allard (1910) who heard it in the spring in northern Georgia.

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stood, however, that the word "cricket" is commonly used for any member of the family Gryllidae.

All other races of field crickets in North Carolina sing with a chirping song like northern field crickets. These races were revealed by differences in habitat and seasonal occurrence. In late April or May a chirping song may be heard in woods at night. The notes are somewhat creaky and issue with an inconstant rhythm, the rate varying from 3 to 5 chirps per second, depending partly on temperature and partly on the "mood" of the singer. These crickets live among the dead leaves in or near woods and seldom sing in daylight. One can ride along a road at night and hear the chirping song while passing woods and the trilling song while passing fields and pastures. Low swampy woods as well as upland oak or pine woods and even the xeric sandhill woods are inhabited by the chirping woods cricket. The song becomes scarce in July and disappears in early August.

In the mountains of North Carolina from the Blue Ridge westward, only one type of field cricket song is heard. This is a loud vigorous chirping which is commonly heard in old weedy fields, clover fields, stony hillsides, and other treeless habitats. It is absent from the woods except where there are natural openings or small clearings. These crickets do more singing during the day than other races, but like other field crickets they are most apt to be silent during the middle of a hot day. This song has been heard every month from May to October.

Along the coast of North Carolina chirping field crickets are common during late summer and fall. They differ from the woods race by inhabiting the flat sandy area bordering the ocean beaches. This may be grassy or shrubby or largely bare sand, but is not wooded. These crickets are very large and usually sing in a leisurely manner with 2 or 3 chirps per second. Crickets assumed to be of the woods race may be heard in early summer in wooded areas which occur farther back from the ocean. Trilling crickets have also been heard near the beaches, especially in inhabited areas where grass is grown for lawns.

On the basis of field observations alone it appears that there are four races or populations of field crickets in North Carolina. The writer has not been able to identify most of these races as any of the old arbitrary species. For convenience of reference it seems desirable to give the races nicknames and to define them by characteristics of the living population as follows:

Triller. The only field cricket singing with a continuous trill. Found in fields and grasslands.

Woods cricket. The only field cricket found in forested areas; also found in shrubby second growth and near forest borders. Chirping song, seldom heard before dark.

Mountain cricket. A chirping cricket found in old weedy fields, stony hillsides orchards, clover fields and other non-forested areas in western North Carolina.

Beach cricket. A chirping cricket found in sandy, treeless areas along the coast.

A few chirping crickets have been heard from August to October at Raleigh and several localities on the coastal plain. Their racial status is uncertain because they do not fit into the known pattern of seasonal or ecological distribution and known range of any of the races. It is possible that the fringe of mountain cricket population extends eastward to the coast or that the beach cricket spreads inland over the coastal plain in reduced numbers. A few such specimens captured in the coastal plain have been of large size like the beach cricket.

Distribution of the Races of Field Crickets

The triller and the woods cricket have about the same distribution in North Carolina. They probably cover the entire state as far west as the foot of the Blue Ridge Mountains. Neither race has been found, within the state, from the

Blue Ridge westward. This area of higher altitude is inhabited by the mountain cricket only, which also extends eastward from the mountains in decreasing numbers to cover more than the western half of the piedmont, reaching points in the vicinities of Chapel Hill and Pittsboro. The beach cricket is known to the writer mainly in the vicinity of Carolina Beach, near Wilmington, N. C. It has also been observed at Smith Island and Holden Beach. Probably it has a much more extensive range along the coast and the shores of the sounds, but field observations are lacking. Chirping field crickets, probably beach crickets, were common in grass and about buildings in the village of Swanquarter on Pamlico Sound, on September 3, 1947. They were not heard there on July 24, 1947, when only trillers were heard. This agrees with what we know about the seasonal history of the beach cricket.

The distribution of these four races of field crickets in other states is very uncertain because field observations on songs, habitats, and seasonal occurrence are lacking. Apparently no observations on the woods cricket have been made outside of North Carolina. The triller is definitely known only in Georgia, Florida and Tennessee, as recorded below. The writer observed trilling crickets on December 11, 1949, on the grounds of a tourist court at Waycross, Ga. He also reared a colony of trillers from a single female captured at Panama City, Fla. The Tennessee records are based on a few trillers heard in lawns in the town of Gatlinburg on June 24, 1947. The trilling song was first observed by Allard (1910) at Thompson's Mills, Ga., about 40 miles northeast of Atlanta. In the more mountainous Towns County, bordering North Carolina, Allard heard only chirping crickets. These were evidently the same as the mountain crickets of North Carolina. The mountain cricket has been heard by the writer at Gatlinburg, Tenn., and in the mountains of Virginia. On June 24 and 25, 1941, chirping field crickets were heard along the highways all the way from Ohio across West Virginia and southwestern Virginia and east of the Blue Ridge Mountains at least as far as Stokes County, N. C. At this time adult crickets would be of the spring brood. The beach cricket has been observed by the writer outside of North Carolina only at Cherry Grove Beach, near the northern end of the South Carolina coast.

Localities in North Carolina where the triller has been observed are listed as follows: Caswell Beach, Holden Beach, Carolina Beach, Wilmington, Lake Waccamaw, Castle Hayne, Burgaw, Wallace, Newton Grove, Goldsboro, Raleigh, Hamlet, Core Point, Belhaven, Swanquarter, Marion and North Wilkesboro.

North Carolina localities where the woods cricket has been observed are as follows: Carolina Beach, Lake Waccamaw, White Lake, Spout Springs, Newton Grove, Raleigh, Eagle Springs, Walkertown, Danbury and North Wilkesboro.

North Carolina localities east of the Blue Ridge Mountains where the mountain cricket has been observed are as follows: Marion, Morganton, Valdese, Statesville, Lexington, Siler City, Pittsboro, Mt. Airy, Danbury, North Wilkesboro, Graham, Kernersville and Chapel Hill.

North Carolina localities where the beach cricket has been found are all mentioned above.

The Races Have Different Seasonal Histories

Information on the seasonal history of field crickets has been derived from three sources, as follows:

1. Field notes on the presence of singing males, thus giving evidence on the seasonal occurrence of the adult stage, from which can be derived the probable periods when eggs and nymphs are present.

2. Direct observation or collection of different life stages in the field.

3. Rearing of crickets in cages. In most cases the cage was a four gallon earthenware jar with two inches of sand in the bottom. Unless otherwise stated the cages were kept entirely in a laboratory which was heated during cold weather.

The conclusions regarding the seasonal histories are shown graphically in figure 1 to permit comparison. The graphs agree with the main facts but are not quantitatively correct. The graph of the mountain cricket applies to the brood which is in the adult stage from May to July. The fall brood, mountain cricket probably has a seasonal cycle similar to the beach cricket but without hibernating nymphs.

The seasonal history of the triller has been closely followed by observations on life stages in the field and by observations on the singing periods of the adults for over twenty years. The singing starts with the first warm nights in March and continues to at least the middle of June. This is followed by one or two weeks during which the song is not heard. In early July the adults of the first complete generation start singing and continue until the middle of September. During warm fall seasons a few adults of the second generation sing in late October or November but most of the second generation hibernate as nymphs and become adults during the following spring.

Further information on the seasonal history of the triller has come from rearing records, as summarized below. A single female was captured and caged in early May, 1947. Young crickets hatched by June 3. They grew rapidly and some became adults of the first complete generation on July 17 after a nymphal period of 44 days. On July 29, some of the young adults of both sexes were placed in another cage. Young crickets of the second generation started hatching on August 11, 1947, after an incubation period of 13 days. A few adults of the

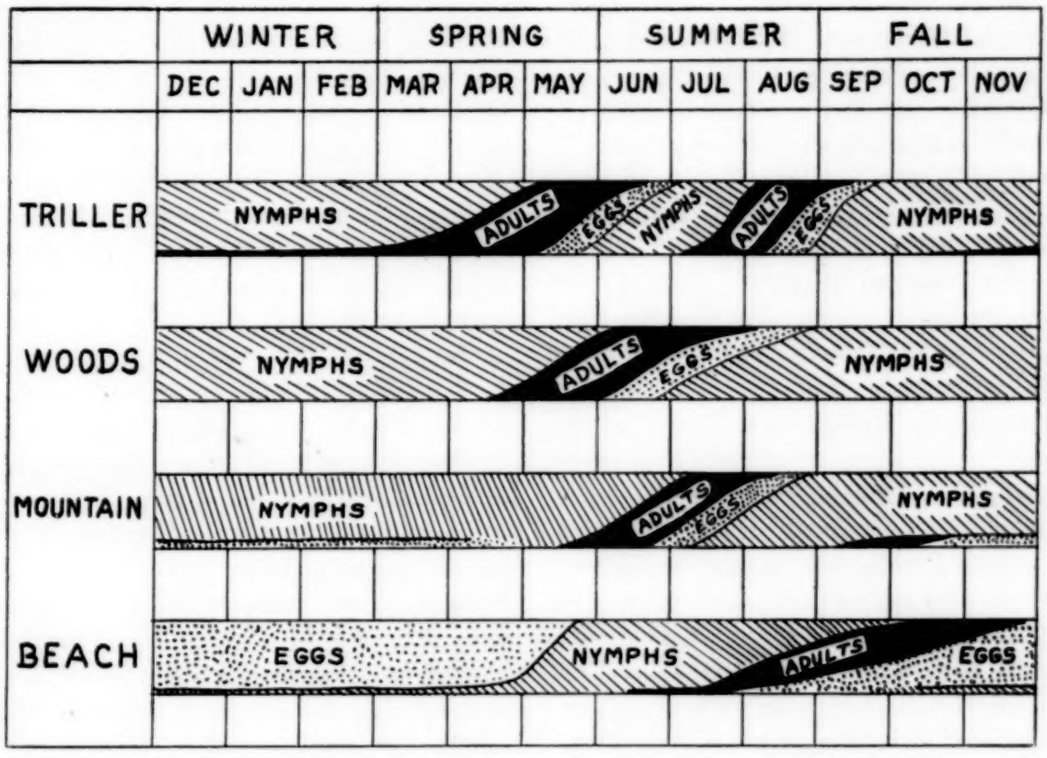

FIG. 1. Seasonal histories of four races of field crickets in North Carolina. The graph for the mountain cricket applies to that part of the population which reaches the adult stage in May and June.

second generation appeared on October 9, showing a total nymphal period for some individuals of about 60 days. However, most of the second generation crickets went through the winter as nymphs, even though the cage was in a heated room.

By April 15, 1948, many of the overwintering crickets had become adults and were depositing eggs. On June 3, young crickets of the first generation were found. Some of these matured by July 28, and young crickets of the second generation started hatching August 12, 1948. Thus for the second year the two generation cycle repeated itself on nearly the same dates. The rearing records agree closely with the cycle observed in the field. The normal cycle was modified very little by keeping the crickets in a heated room. The few adults singing in late fall out of doors probably hibernate as the nymphs do, and may be among those heard singing in March. Some late fall adults reared in the laboratory were paired on December 9, 1947, and produced young by February 20, 1948. The writer has seen no evidence that eggs are laid out of doors in the fall.

Information on the seasonal history of the woods cricket is also based on many years of field notes. The singing period starts with adults appearing in late April, from nymphs that passed the winter under dead leaves in the woods. The singers are most numerous in late May and June but the song is still heard until late July or early August. During fall and winter only nymphs are found in the woods.

Rearing observations on the woods cricket started on June 18, 1947, with adult crickets collected in the middle of a woods. Three pairs were caged. Young crickets appeared on July 8 after an incubation period of 20 days. All of the female adults were still alive on August 20 but the last male died about August 1. On August 26 all crickets in the three cages were found dead, due to high temperature or an invasion of ants. The study was continued a few days later by collecting a number of nymphs from the same woods where the original adults were taken. They varied in length from 8 to 15 mm. and were about the same size as the ones that died. These passed the winter as nymphs although kept in a heated room. The first adult was recorded on March 30, 1948, which was about a month earlier than normal for the race. By April 15, several adults had appeared in the cage and by June 15 young started to appear. These nymphs grew as slowly as those of the previous year and by November 4, 1948, they were mostly about 15 mm. long. No adults had appeared by March 18, 1949. Most of this lot matured in April and May, thus completing two cycles of one generation per year and agreeing very closely with events in nature.

The song of the mountain cricket has been heard during every month from May to October. Field crickets do not live this long in the adult stage, so it is reasonable to assume the existence of two seasonal broods as in the northern states, but field observations for midsummer are lacking and we do not know whether there is a period between broods when adults are scarce or absent. Field observations have been more extensive in spring and early summer. At Glenville in the southern part of North Carolina at an elevation of about 3,500 feet, the mountain crickets did not start to sing before May. In April large nymphs were observed, but no adults. By June adult crickets were fairly numerous, and there are a large number of June records of the song in other parts of the mountains and in the piedmont areas inhabited by this race. After July 3, 1941, when adult crickets were noted as common in the northern North Carolina mountains, there are no more observations until August 16, 1950, when chirping crickets were very scarce near Hendersonville. On August 19, 1945, they were heard along the roadsides from Boone to Asheville. Other field records are September

9, 1948, and September 20, 1945, when numerous crickets were heard singing, both in the upper piedmont and in the mountains. The latest seasonal record is October 9, 1941, when the crickets were heard, but not in great numbers near Blowing Rock. By turning over loose rocks, two adult males were caught, but adults appeared to be less common than half grown nymphs.

A number of partial seasonal histories of the mountain cricket were obtained, over a period of years, by caging adult crickets collected in June. Five lots were collected at different points in the North Carolina Mountains and one lot from Gatlinburg, Tennessee. Young crickets started to hatch within 15 to 27 days after the adults were caged. All of the parents died in July or early August. With two lots no further observations were made. With two other lots, from Highlands and Weaverville, N. C., the young crickets were kept for a longer period but none of them matured by December. Under natural conditions they would all have hibernated as nymphs. The fifth lot, from the Blue Ridge Mountains east of Jefferson, N. C., had a small number of offspring which matured in September, although the great majority went into the winter as nymphs. All the early adults and a large number of nymphs were placed in an outdoor cage early in October. Both adults and nymphs were observed on November 4, but when the cage was opened the following April, only one nymph could be found. The others had died or escaped and it was not known whether the adults had deposited any eggs. The crickets from Tennessee also had a few offspring mature in September. Two pairs of these early adults were segregated on September 12 and kept in the laboratory. Their offspring started to appear on October 20. It is doubtful whether eggs deposited as late as these would hatch under natural conditions. The last two records suggest the possibility that a small proportion of the offspring of the spring brood adults may, under natural conditions, mature early enough to join the fall brood. Such individuals, however, did not appear numerous enough to account for the whole of the fall brood, but further investigation is needed to clear up the relationship of the two broods.

Only one attempt was made to rear any of the fall brood of adults from the mountains. A single pair, captured September 9, and caged September 11, died sometime in October. A large number of eggs were deposited, but after the adults died the cage was overlooked and the sand dried. Later it was discovered and kept moist and in the laboratory for the winter. Three young crickets were found on January 16 but no more appeared. If this female had deposited eggs on Grandfather Mountain, where she was captured, it is very probable that the eggs would have remained unhatched during the winter.

The mountain cricket was the most difficult to rear in the laboratory during the winter. Mortality was high and many developed stunted abnormal wings, especially the males. Some wings also appeared to have been chewed off by other crickets.

Evidence on the seasonal history of the beach cricket indicates that the adults are most abundant in late summer and fall and that the winter is passed mainly in the egg stage. The eggs hatch in spring and there is only one generation per year. A few individuals get out of step with the main population as shown by field notes on chirping males. In the vicinity of Carolina Beach, the earliest noted was May 13 when one cricket was heard. One or two crickets were heard on two occasions in June. Even in July only a few beach crickets have been heard.

On April 28, 1948, the only beach crickets that could be found near Carolina Beach were first or second instar nymphs. On July 23, 1947, a large number of medium sized and large nymphs

were found but only two adults, both males. On August 18, 1948, nymphs still outnumbered the adults but a considerable number of males could be heard singing. On October 20, 1948, only two nymphs were seen but a large number of adults were observed or captured, the females greatly outnumbering the males. At Cherry Grove Beach, South Carolina, on August 1, 1946, both large nymphs and adults were present, the former more abundant.

Rearing records of the beach cricket agree largely with what has been observed in the field, but show also that this race may be somewhat more plastic in its seasonal history and more readily influenced by changed conditions. Laboratory rearing started with two adult males and a number of nymphs collected on July 23, 1947. The first female adult appeared about August 20 and the last matured September 6. The last surviving adult, a female, died about October 31. Offspring appeared September 15 and again in December, February and April from the same lot of parents. The first adult cricket of the new generation matured on March 23, 1948. By April 17, several adults were present. These had been growing since September 15. Later hatched nymphs continued to mature until the last of July.

Under outdoor conditions there would have been no hatching in December or February, but eggs could have hatched in September or April when outdoor temperatures are high. If September nymphs could survive the winter, they probably would mature ahead of those hatching in April and might explain the few early summer adults heard singing on several occasions.

Another record was kept of the rearing of three small nymphs collected at Carolina Beach on June 10, 1948. The first adult (male) appeared on August 12 and the others, including one female, matured a little later. By October 20, no offspring had appeared and the jar containing the sand, with deposited eggs, was moved out of doors, where it remained all winter partly buried in the soil. Young crickets started to hatch on April

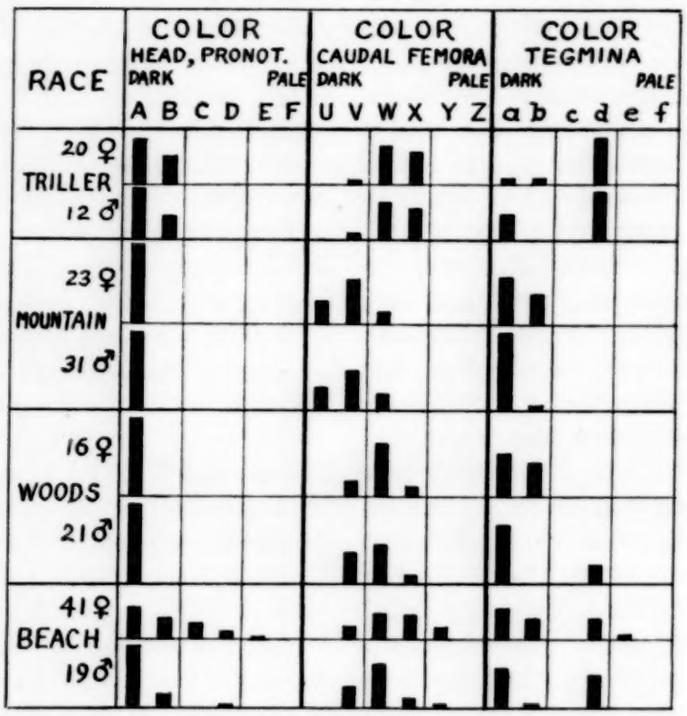

FIG. 2. Proportions of the four races of field crickets falling into the color classes of Rehn and Hebard.

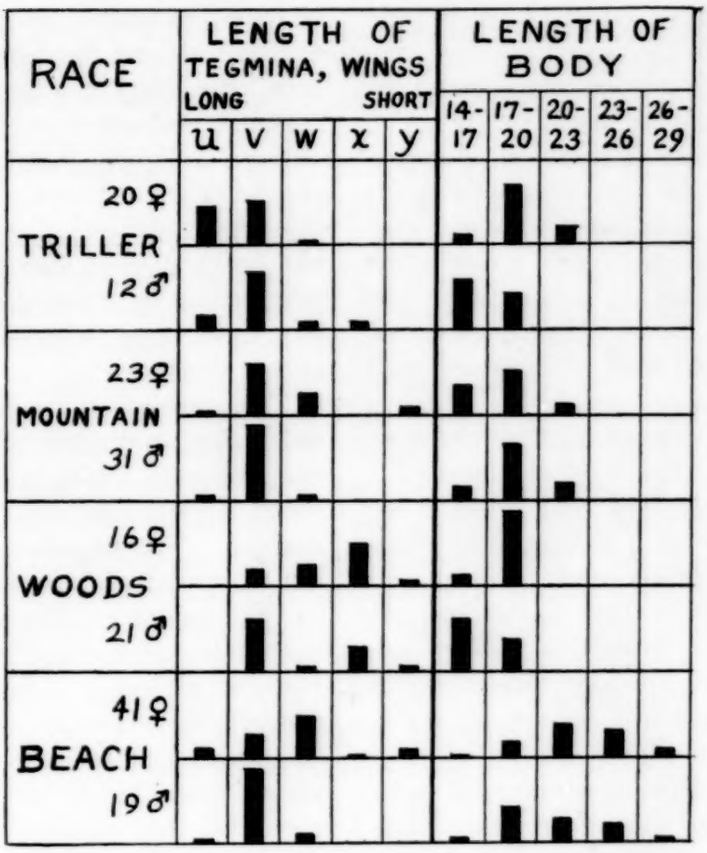

FIG. 3. Proportions of the four races of field crickets falling into the classes of Rehn and Hebard based on length of wings and tegmina; and five body length classes in millimeters.

21, 1949, and the first adult appeared July 25. The others matured in August and September. This rearing record agrees closely with observations in the field.

On October 20, 1948, two large females collected at Carolina Beach were caged in the same jar. They deposited eggs which hatched in the laboratory from February 24, 1949, until April. The duration of the egg state is shortened by being kept in a warm room in winter.

ANALYSIS OF THE RACES ACCORDING TO THE CLASSES OF REHN AND HEBARD

In order to find whether it would be possible to define the races of field crickets by visible characters the collections of specimens known to be of a certain race were analyzed according to the classes devised by Rehn and Hebard (1915). According to this scheme each specimen is given a set of five symbols according to the observer's personal interpretation of its place among the several defined classes of five different sets of characters. These classes will not be defined here except to name the characters and the complete range of variation.

A to F—Coloration of head and pronotum. Black to pale.

U to Z—Coloration of caudal femora. Black to buffy.

a to g—Coloration of tegmina. Unicolorous dark to unicolorous very pale.

u to z—Macropterism and brachypterism. Tegmina large, wings fully developed to tegmina (and wings) greatly reduced.

1 to 5—Length of body. Very large to very small. In place of Rehn and Hebard's symbols for body size, these five classes are here defined by length in

millimeters, starting with 14 to 17 and ending with 26 to 29.

The individual classes are defined by Rehn and Hebard in such terms as to permit considerable latitude in interpretation. It would be nearly impossible for two observers to analyze a series of specimens and come out with exactly the same set of symbols. If the same observer analyzed more than one series, however, he would tend to interpret each series by the same standards. Differences between series would then show up as differences in the symbols used.

After each specimen was given a set of 5 symbols the next step was to count the number of times each symbol was used for each race and determine the percentage of the collection falling into each class. This was done independently for each sex. The results are shown in figures 2 and 3. The height of the bars indicates the percentage of the total number of specimens falling in each class. A single tall bar means that all specimens of that race and sex are alike for that character. Several short bars indicates that the race exhibits great variability for that set of characters. The number of specimens examined for each race and sex is shown at the left. The number is small in some cases because only specimens known to be of a certain race could be used. Many had been reared in the laboratory.

The results show plainly that all four races have one or two classes in common for each group of characters. The differences between races are only differences in the percentage of the population falling into the various classes. No race is sufficiently well defined by any combination of characters to make it possible to identify most specimens. If the four collections were scrambled after removing the labels, it would be impossible for anyone to sort them out again, although a few specimens probably could be correctly placed, such as large pale specimens of the beach cricket.

Although the races cannot be defined in terms of visible characters, a few generalizations can be drawn. In the triller race, the largest class in the color of tegmina was interpreted as class d, "unicolorous, slightly pale." This is the most characteristic feature of the triller, but it does not hold true for all specimens and the beach cricket also has a rather large proportion in class d. The triller also has more long winged individuals than other races.

The mountain cricket has the least pale color on the caudal femora and is the only race in which solid black hind femora appear among the specimens examined. The tegmina average the darkest in the mountain cricket.

The woods cricket is very close to the mountain cricket in the color of the tegmina although a larger proportion of individuals show a wider reddish area on the caudal femora. No long winged forms were found among the 37 specimens of the woods cricket. This race also has a higher proportion than other races in class x "tegmina slightly reduced, wings very much reduced."

The beach cricket shows more variability in color, with a large proportion in the paler classes, although many dark individuals are also present. This race has the largest average size but is also represented by a few individuals in the smallest size class.

Crossing Experiments

Having discovered that there are four populations of field crickets in North Carolina the question arose as to how much racial interbreeding takes place in nature. There are times and places where the adults of different races may come in contact and where hybridization might possibly occur. Crossing experiments were planned to see whether hybrids could be produced by caged crickets.

The first crossing experiments were with the triller and the woods cricket, both being plentiful around Raleigh. Adult virgin females of both races were obtained by segregating female nymphs

which are easily recognized in the later stages by the protruding ovipositor. After the virgin females matured they were paired with known adult males in glass jars containing moist sand. They were provided with food and water until they died. The sand was kept moist for periods varying from 7 weeks to 4 months to give the eggs a chance to hatch if they were viable. Usually one female of each lot was paired with a male of her own race. Such pairs produced offspring after periods varying from 13 days to about a month depending mainly on the age of the female at the time of pairing.

No offspring were obtained from a total of 19 crosses of the triller and the woods cricket. Later the experiments were extended to include all four races by pairing one or both sexes of each race with the opposite sex of the other three races. The results are shown in figure 4. A total of 50 attempts to hybridize different races gave negative results. On the other hand, out of 25 cases where a cricket was paired with its own race, only two failed to produce offspring. In one of these the male apparently failed to inseminate the female, as revealed by post-mortem examination of the spermatheca. In the other case both sexes were defective specimens, entirely lacking tegmina and wings, from an overcrowded cage.

In several of the matings where a triller was paired with its own race, the male and female were from widely separated localities in North Carolina. Raleigh trillers were paired with trillers from near Wilmington, Lake Waccamaw and Swanquarter and all produced offspring. Offspring were also obtained when a North Carolina triller was paired with a descendant of a female cricket from Panama City, Fla., whose sons sang with the trilling song.

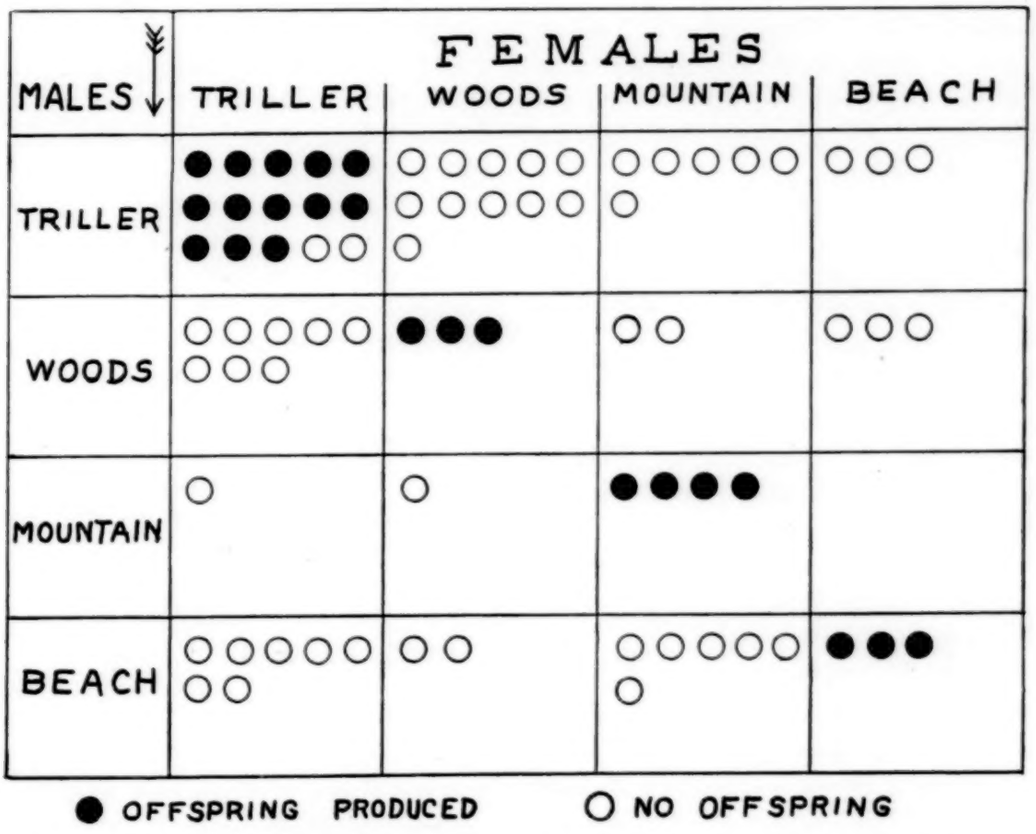

FIG. 4. Results from segregated pairs of field crickets of four races. Each pair is represented by a circle, solid black if offspring were produced and white if no offspring were produced.

In another series of experiments, seven males were given a choice of mating with a female of their own race or of some other race. Each male was confined with two marked virgin females. The females were killed after several days and their spermathecas were examined. In three cases the female belonging to the same race as the male had sperms while the other female had no sperms. In one case the female of the same race had sperms but the other female had been devoured. In the three other cages no sperms were found in either of the females. The result of these experiments as well as the observed behavior of certain males, suggest that the males may show some preference for females of their own kind. Such psychological isolation, if it exists at all, is not strong enough to prevent a male from mating with a female of another race when not given any other choice. In four instances females were observed with a spermatophore which came from a male of another race. Three females, which were paired with a male of another race, were killed and dissected for evidence of insemination. All of them had sperms in the spermatheca.

The failure to produce hybrids by crosses of different ecological races of field crickets apparently is not due to lack of insemination nor is it due to a failure to produce and deposit eggs. Virgin females and females confined with males of another race usually deposited a few eggs but seldom more than a hundred. The bodies of such females usually became more distended with eggs than females paired with their own race. One virgin beach female had 975 fully developed eggs in her ovaries.

The foregoing experiments and observations may not be extensive enough to prove that hybridization between races of field crickets never occurs, but at least they show a high degree of reproductive isolation due to a failure of the eggs to become fertilized by sperms of another race or to a failure to produce a viable zygote.

It is probably useless to speculate on the origin of the field cricket races. Ecological conditions must have changed greatly from the wilderness habitats in which the crickets evolved. The triller, woods cricket and beach cricket are evidently isolated from each other by habitat. It is not unreasonable to assume that the original isolation was along ecological lines. The mountain cricket and the triller, however, both occur in fields in the central and western piedmont. Rather limited observations in this region show the triller to be more partial to grass than the mountain cricket which is most often heard singing in weedy fields. The mountain cricket may prove to be a northern geographical race.

The evidence presented in this paper seems to show a degree of reproductive isolation between the four races of field crickets as complete as between true species. Speciation has not proceeded along morphological lines to the point where the races can be identified by visible characters. The writer does not believe that anything is to be gained at the present time by giving the races scientific species names. Old names formerly used would still be available and any attempt to identify them with the races as here defined would lead to as much confusion as former attempts to split the American field crickets into species. Until the number and distribution of such races is better known over a wider area, it is better to use the proposed nicknames, which are not subject to the rules of nomenclature.

Summary

Early attempts to split the genus *Acheta* (*Gryllus*) into species resulted in much confusion. The works of Lutz (1908) and Rehn and Hebard (1915) showed that the characters used for separating the indigenous New World species were too variable to be of any value. All species were reduced to synonyms

under the oldest valid name, *A. assimilis* Fab.

The present study shows that this is an over-simplification and that *A. assimilis* does not conform to the present day concept of a species as an interbreeding population. Even within the limits of the state of North Carolina, four races or populations have been recognized, which differ by such characters as song, seasonal history and choice of habitat.

A study of certain color and structural characters showed some statistical difference between races, but no visible character or combination of characters was found which would make it possible to identify specimens.

When males and virgin females of different races were paired, mating occurred and eggs were laid but no offspring appeared. A total of 50 attempts to hybridize different races all gave negative results. Pairs of the same race rarely failed to produce offspring. Some intrinsic isolating mechanism apparently exists, which prevents fertilization or the formation of a viable zygote.

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VARIATION IN A LOCAL POPULATION OF *PEMPHIGUS* [1, 2]

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INTRODUCTION

The purpose of this paper is to present a study of the variation of morphological characters within a local population of the aphid *Pemphigus populi-transversus*. Twenty-one characters are analyzed statistically with a view to selecting those representative of genetic variation within the population. The characters thus selected are to be used for a study of intraspecific variation. Certain material on character interrelations is included since it is readily derived from the same data.

Much work has been done in recent years on the analysis of intraspecific variation in both animals and plants. To the knowledge of the writer none of the work on animals, with the exception of that of Kinsey (1930, 1936) on *Cynips*, treats of partially or fully asexual or parthenogenetic forms. Kinsey's results did not demonstrate any particular deviation in population structure for species of this genus with alternately sexual and agamic generations. As the main emphasis of his work was not a detailed analysis of intraspecific characters, these results cannot be considered as definitive.

For this reason the author thought it profitable to examine a genus of a partially parthenogenetic form to learn what character variation and population structure in such a group would be like. The choice fell on the aphid genus *Pemphigus* Hartig,[4] gallmakers on the petioles, leaf blades and shoots of poplars, for the following reasons: *Pemphigus* has several parthenogenetic and only one sexual generation per year. The offspring of the parthenogenetic generations are very numerous, and thus there is opportunity for studying variation within and between clones as well as for observing the effects of any rapid buildup of favorable mutations and genic combinations through parthenogenesis. The galls are plentiful on poplars, which themselves are common and easily accessible trees throughout most of the United States. They are simple to collect and store in quantity, while the aphids are relatively easily prepared for study.

The success of any study of variation depends to a large extent on the prudent choice of the characters to be studied. This applies equally well to work in taxonomy, speciation and genetics. We are constantly in quest of the "good" character. Unfortunately all too often "good" characters are chosen on a subjective or arbitrary basis and at other times earlier work has set a precedent that is followed without criticism. In the present study it was decided to attempt to find characters on a more or less objective and precise basis. A subsequent paper will deal with the variation of *P. populi-transversus* in eastern North America.

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[1] Part 1 of a study of variation in the aphid genus *Pemphigus*.

[2] This paper is part 1 of a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the Department of Zoölogy of the University of Chicago.

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[4] It has recently been shown (see Laing, F. 1951. Ent. Monthly Mag., **87**: 108) that the genus name *Byrsocrypta* Haliday has priority over *Pemphigus*. In the present paper the well known name *Pemphigus* has been retained.

Biology of *Pemphigus populi-transversus*

This aphid forms galls on *Populus deltoides* and perhaps on *P. sargentii*. Published records of other host species of *Populus* are doubtful. Its range is roughly from the northern border of the U. S. to northern Florida, and from the Atlantic seaboard to an as yet undetermined western limit, near the longtitude of Kansas or Colorado, or perhaps as far as California.

The annual cycle starts in spring with the stem mother or fundatrix which develops from an overwintered egg that is the product of sexual union. The fundatrix is therefore diploid. As a first instar nymph she wanders around the branches of a cottonwood tree until she settles on the petiole of an unfurling leaf, where she begins to suck. As a result of her activity there develops a growth on the petiole which eventually becomes a hollow, globular or ovoid gall with a transverse, slitlike opening—hence the species' name. After maturity the stem mother gives birth parthenogenetically to other parthenogenetic females that are winged. The latter are also diploid and presumably isogenic with the stem mother. These alates, known as fundatrigeniae, leave the gall and find their way to some secondary host. A number of cruciferous and composite herbs serve as such hosts (see Jones and Gilette, 1918).

On the secondary host a new apterous generation of females is produced by the alates. These forms are still diploid and members of the same clone. They migrate to the roots of the secondary hosts where they spend summer and early fall producing parthenogenetically an indefinite number of generations of females. They are known as alienicolae.

Around October (near Chicago) there appear new forms, called sexuparae, produced by the alienicolae. These are alate parthenogenetic females, different in appearance from the alate fundatrigeniae and with a progeny fixed at six sexual individuals, four females and two males. The sexuparae are still diploid and direct clonal descendants of the stem mother. They emerge from the ground and fly back to the cottonwoods where they give birth to their six offspring. These, the sexuales, have a chromosome number of 2n for the females and 2n-1 for the males. The sexuales are nymphal in appearance, with rudimentary mouthparts, and each female lays a single egg only. The males produce two kinds of sperm, n and n-1, with only the former viable. The egg is deposited in cracks in the bark and overwinters to develop into a stem mother during the following year. From Jones and Gilette (1918) it appears that in the South the sexuparae may overwinter on the secondary host roots and remigrate to *Populus* in the spring.

For this study only aphids present in galls, i.e. stem mothers and alate fundatrigeniae, were used and the term "alates" whenever employed refers to such winged forms only.

The Choice of Characters

Leaving aside physiological or biological attributes which were not to be investigated, what kind of character would it be desirable to choose for the particular study intended? Mayr (1942, p. 20) defines a taxonomic character as ". . . any attribute of an organism (or better of any group of organisms) by which it may differ from other organisms." This definition does not appear specific enough for a study of intraspecies variation. All characters useful in an intraspecific study must vary for at least part of the range of the species, and the variation must at least in part be initiated by genetic factors.

A useful character may vary little or not at all in a local population, due either to fixation of its genetic factor or factors coupled with relative resistance to environmental influences, or may appear invariable due to lack of refinement in measurement or observation. In the

former case this character might have different, perhaps equally invariant phases in other local populations, with some populations being of a mixed composition. This would be the basis for a polymorphic character. When graphed it might give a binominal or polynominal frequency diagram or, if the phenotypic expression of the genetic factor varied somewhat, a bimodal or polymodal frequency distribution.

Other useful characters might vary in a local population in the manner of the normal curve or some deviation from it due to a multiplicity of genetic factors that are not fixed for the population, or due to a variety of environmental influences impinging on it, or most likely for both of these reasons. The situation may be, and frequently is, complicated by intergradations between, and superimpositions of, the two types of characters.

It is almost a hopeless task to unravel the tangle of genetic and environmental factors that underlie the expression of most morphological characters. Progress has been made in a number of ways, such as the analysis of guinea pig coat color through extensive breeding experiments (Wright, 1920), or the analysis of chaetae number in *Drosophila* through mass selection and factorial analysis with markers (Mather and Harrison, 1949).

For the evolutionist whose main emphasis is not on the field of genetics it must suffice that the character chosen owes its variation in part to variation in genetic factors. That this is no easy task is obvious since the separation of genetic and environmental causation often resists solution. The writer will refrain here from a discussion of the various analytical approaches to this problem and merely present his own endeavors in this line which, however, are adaptable to the rather limited case of clonal animals only.

Material and Methods

The population sample of *Pemphigus populi-transversus* chosen for this study was collected along U. S. Highway No. 52, 4 m. S.E. of Ripley, Brown County, Ohio, on September 9, 1949 (Lot No. 49–59). This locality is situated in the Ohio River flood plain. Collections were made from a single tree. Only those galls containing at least 4 alates were used for the study. There were 28 such galls among a total of 46 collected galls. The 4 alates per gall, a total of 112 alates, were mounted on slides and measured for 21 characters each, 15 exhibiting continuous variation and 6 meristic. The following measurements were taken:

1. Total length— from anterior edge of head to tip of abdomen (dorsal view)
2. Forewing length— from base of wing to distal end of stigma, measured along costa
3. Head length— from anterior to posterior edge of head (dorsal view)
4. Head width— distance between lateral ocelli
5. Thorax length— from anterior edge of mesothorax to posterior edge of metathorax (dorsal view)
6. Thorax width— widest frontal plane through pterothorax
7. Femur length— length of metafemur
8. Tibia length— length of metatibia
9. Tarsus length— distance from proximal end of second metatarsal segment to base of claws
10. Rostrum length— from base of labium to tip of rostrum (ventral view)
11. Last segment of rostrum length—
12. Antennal segment III length—
13. Antennal segment IV length—
14. Antennal segment V length—

15. Antennal segment VI length— from base of segment VI to tip of unguis
16. Number of sensoria on antennal segment III—
17. Number of sensoria on antennal segment IV—
18. Number of sensoria on antennal segment V—
19. Number of sensoria on antennal segment VI—
20. Number of sclerotic islands in primary sensorium of antennal segment V— Only free "floating" islands bearing at least one seta were counted
21. Number of sclerotic islands in primary sensorium of antennal segment VI— Only free "floating" islands bearing at least one seta were counted

In 25 of the 28 galls, stem mothers were obtained. These were mounted and measured as well. The following eight continuous characters were studied:

1. Head width— distance between lateral ocelli
2. Femur length— length of profemur
3. Tibia length— length of protibia
4. Tarsus length— distance from proximal end of second protarsal segment to base of claws
5. Antennal segment III length—
6. Antennal segment IV length—
7. Last segment of rostrum length—
8. Total length— from anterior edge of head to tip of cauda, regardless of whether this is or is not the hindmost part of the body

Without special and time-consuming methods of preparation the limits between head, thorax and abdomen are hard to find in a stem mother, which latter is practically an undifferentiated bag of embryos on three pairs of tiny legs. Thus head length and thoracic measurements could not be taken. The foreleg of the fundatrix was measured rather than the hind leg as in the alates, since this was more accessible and less often damaged. The last segment of the rostrum, rather than the entire structure, was measured since the exact limits of the latter are hard to determine in the fundatrix. There are only four antennal segments in the fundatrix; hence no measurements corresponding to lengths of antennal segments V and VI in the alates were possible. There are also no annular sensoria in the stem mother corresponding to those of the alates.

The Characters Studied Singly

A compilation of the measurement statistics and the analysis of variance of the characters of the alates can be found in table 1. Column 1 consists of the means and their standard errors based on the individual measurements of the 112 alates. However, basing standard errors on the number of alates is not quite justified. We are mainly interested in the variation among the genotypes present and the four alates per gall are just different expressions of one genotype. By an extension of this procedure we might measure fifty alates each for our 28 galls if these were available, have a total $N = 1400$ and reduce our standard errors considerably. Yet our means as such would be little more reliable with respect to those of the total population. We are thus dealing with presumably 28 genotypes and in column 2 of table 1 are found the standard errors of the means based on variance of gall means and $N = 28$.

Columns 3 and 4 show the coefficients of variation with their standard errors, those in column 3 based on $N = 112$ and the standard deviation of the individual

TABLE 1. *Measurement statistics and results of analysis of variance for 112 alate Fundatrigeniae*

Character	$\bar{X} \pm \sigma_{\bar{X}}$	$\pm\sigma_{\bar{X}}$ (N = 28)	C.V. $\pm\sigma_{C.V.}$	C.V. $\pm\sigma_{C.V.}$ (N = 28)	Analysis of variance P	% of variance determined by intragall factors
Total length	2.06 ±.032	±.058	16.3± 1.12	14.9± 1.99	+++	24.6
Forewing length	2.06 ±.021	±.036	10.7± .72	9.3± 1.24	+++	34.9
Head length	.2045±.00227	±.0036	11.7± .78	9.3± 1.24	++	61.0
Head width	.3786±.00293	±.0050	8.2± .55	7.0± .93	+++	37.9
Thorax length	.574 ±.0067	±.0126	12.4± .83	11.7± 1.56	+++	17.2
Thorax width	.613 ±.0077	±.0141	13.3± .89	12.2± 1.63	+++	24.2
Femur length	.495 ±.0052	±.0092	11.2± .75	9.9± 1.32	+++	31.2
Tibia length	.680 ±.0075	±.0130	11.7± .78	10.1± 1.35	+++	35.5
Tarsus length	.1489±.00100	±.00157	7.1± .47	5.6± .74	+++	52.1
Rostrum length	.2971±.00215	±.00274	7.7± .51	4.9± .65	++	79.8
Last rostral segment length	.0663±.00039	±.0039	6.2± .42	3.1± .41	−	100.0
Antennal segment III length	.1690±.00184	±.00296	11.5± .77	9.3± 1.24	+++	48.3
Antennal segment IV length	.0721±.00077	±.00122	11.3± .75	8.9± 1.19	+++	51.3
Antennal segment V length	.1005±.00092	±.00132	9.7± .65	7.0± .93	+++	65.8
Antennal segment VI length	.1722±.00116	±.00181	7.1± .48	5.5± .74	+++	53.3
Sensoria number on segment III	3.71 ±.080	±.072	22.8± 1.64	10.2± 1.37	−	100.0
Sensoria number on segment IV	.21 ±.040	±.039	208 ±43	100 ±23	−	100.0
Sensoria number on segment V	1.045 ±.0234	±.0225	23.7± 1.67	11.4± 1.52	−	100.0
Sensoria number on segment VI	1.045 ±.0196	±.0184	19.9± 1.38	9.3± 1.25	−	100.0
Islands number on segment V	2.21 ±.067	±.063	32.3± 2.37	15.1± 2.07	−	100.0
Islands number on segment VI	2.88 ±.106	±.111	38.9± 2.96	20.4± 2.92	−	96.8

All linear measurements are in mm. For explanation see text.

alates, while those in column 4 are again based on N = 28 and the standard deviation of the gall means. We note that the coefficients in column 4 are lower than those in column 3 throughout, a good deal of individual, non-hereditary variation having been eliminated by the use of gall means for each character. It will be noted, however, that the values for C.V. are rather higher than usual for comparable material. This results from the fact that the population is more or less strongly skewed to the left for the continuous characters with some indication of a small second mode at the lower end of the distribution. Other populations of *P. populi-transversus* from Illinois demonstrate similar high coefficients of variation. The C.V. for the sensoria number for antennal segment IV has the remarkable value of 100, this character showing a highly anormal frequency distribution. In fact, most individuals have no sensorium, only a few have one, and but one aphid has two sensoria. The observed frequency distribution agrees well with a Poisson distribution of the same mean.

The measurements for each character were then subjected to an analysis of variance to investigate whether the variance of each character was based on individual variance of the alates within galls only,

or whether there was present an extra component of variance based on variance between galls. Since all aphids within a gall are presumably isogenic, any intragall variance must be due to variation in the external and internal environmental factors affecting each member of the clone. As possible factors of this kind we might list variations in intragall microclimate, success in feeding, accidents such as disturbance by other aphids or predators, some effects of the temporal sequence of birth, internal physiological factors during development, etc. Whatever these factors are it should be reëmphasized that they are non-hereditary.

Unfortunately intergall variance cannot be assumed to result from genetic factors alone. All environmental influences varying from gall to gall will contribute to this variance as well. Obvious environmental factors of this kind would be differences in microclimate from gall to gall resulting from variable exposures to sun and wind. Others might be differences in leaf size and in nutrition. Only refined breeding experiments, keeping these and other factors constant could distinguish between influences of this nature and variance due to genetic variation. These are impractical in an aphid with as complicated a life cycle as *Pemphigus*.

Yet it must be assumed that, unless the population is extremely inbred and homozygous, some of the intergall variance for some of the twenty-one characters chosen would be due to differences in genotype. Few populations of *Pemphigus* are less likely to be inbred than this one from Ripley, Ohio. It is part of a continuous population chain infesting the unbroken salient of cottonwoods in the Ohio River valley. No correlation has so far been found between aphid characters and size or shape of gall, or density of alates within the gall at the moment of collection. A more refined analysis will have to await the assembly of more numerous data. It is the author's assumption that part of the intergall variance is due to variations in genotype. This seems more justified than the assumptions of many authors who, without further analysis, hold that differences between individuals of local populations of animals that are not well known genetically are partly hereditary. I have felt hesitant, because of the objections mentioned above, to call characters exhibiting significant variance between galls genetically influenced, and shall merely call them affected by intergall factors. I do believe, however, that genetic differences enter into their variance and that they are therefore suitable for a speciation study.

Column 5 in table 1 shows the results of the analysis of variance. The P value for the variance ratio F is shown as +++ for $P \leq .001$, ++ for $.001 < P \leq .01$, and −−− for $P > .05$. There is a surprising dichotomy in the data. All continuous characters exhibit highly significant intergall variance, with the exception of the length of the last rostral segment. This is a poor character as its limits were often hard to define. The same can be said of head length and rostrum length which show a lower level of significance than the rest of the characters. These three characters also suffer from an unfortunate choice of scale of magnification which probably caused the depression in their coefficients of variation.

On the other hand none of the meristic characters show any significant intergall variance. Thus we may say that sensoria and island numbers on the antennae do not vary because of genetic variation in the population studied. The sensoria number on segment IV apparently varies at random, as the Poisson-like frequency distribution would lead us to believe. This absence of genetically caused variation is interesting since the number of sensoria on antennal segments is a reliable specific character in the genus, and is also significantly different in the sexuparae of the same species, the forms morphologically most closely related to the alate fundatrigeniae. Apparently the

genetic factors determining sensoria number for the species do not vary within this local population. A study of other populations will demonstrate whether these factors vary within the species at all.

From the variances calculated for the analysis of variance we can obtain σ^2_μ, an estimate of the true variance of means, or in our case, of the true intergall variance.[5] Having an estimate of this variance, we can then get estimates of the percentages of the variance determined by intragall and by intergall factors (see statistical appendix, note 2). Column 6 in table 1 lists the percentages of the variance due to the definitely non-hereditary influences within galls. They range from 17 to 80 per cent for characters with a significant analysis of variance, and are 100 per cent or thereabouts for the other characters. Thorax length, thorax width and total length are the three characters with the largest portion of potentially genetic variance. This ties in well with subsequent results.

We are thus left with 14 characters, since the 6 meristic characters and the length of the last segment of the rostrum show no significant intergall variance. Fourteen characters are still too many to measure for each individual if a variational study of considerable extent is to be undertaken. How can we narrow these down to a total of 3 to 5 characters most representative of the variation pattern of the species?

The Characters Studied in Combination

The total correlations

Current genetic theory asserts that most morphological characters are not entirely determined by specific genes or sets of genes, but that these genetic factors influence series of characters, perhaps through the effect of the genes on a basic metabolic process that would affect a number of organs or regions of the body. A relation of this nature, unless masked by other factors, should result in correlation between the structures concerned. This relation is not confined to genetic influences. Environmental ones affecting more than one character through some mechanism may result in correlations as well. On the other hand, genetic and environmental factors specific to certain characters would tend to dilute and obscure the above-mentioned correlations.

In this particular study three types of significant, non-spurious correlations may occur:

1. Correlations of two characters, both exhibiting significant intergall variance. Such correlations may be due to common intragall environmental influences, common intergall environment, common genetic factors or any combination of these three.
2. Correlations of one character exhibiting significant intergall variance with another exhibiting intragall variance only. Such correlations can be due to common intragall environment only.
3. Correlations of two characters, both exhibiting intragall variance only. Such correlations are due to common intragall environment exclusively.

For the purposes of this investigation correlation types 2 and 3 are of no import. The problem consists of identifying those correlations of type 1 that are caused by common intergall factors, which latter are believed to include genetic factors.

Table 2 shows the total correlations between the 21 characters for the 112 alates. We find that correlations among the meristic characters, which as we have seen exhibit no intergall variance, are not significant with the exception of four correlations, and that no correlations between characters with intergall variance and those without are significant. Thus there are no type 2 correlations and very

[5] Simple statistical procedures will not be illustrated in this paper. They can be found in any of the books on statistics or biometry such as Snedecor (1946). Some of the less common procedures are collected in a "Statistical Appendix" at the end of this paper. For σ^2_μ see note 1 therein.

TABLE 2. *Total correlations between alate characters*

	Total length	Forewing length	Head length	Head width	Thorax length	Thorax width	Femur length	Tibia length	Tarsus length	Rostrum length	Last rostral segment length	Antennal segment III length	Antennal segment IV length	Antennal segment V length	Antennal segment VI length	Sensoria number on segment III	Sensoria number on segment IV	Sensoria number on segment V	Sensoria number on segment VI	Islands number on segment V	Islands number on segment VI
Total length	XXX																				
Forewing length	.68	XXX																			
Head length	.61	.46	XXX																		
Head width	.80	.63	.53	XXX																	
Thorax length	.79	.79	.51	.78	XXX																
Thorax width	.85	.75	.56	.81	.92	XXX															
Femur length	.72	.73	.50	.66	.85	.81	XXX														
Tibia length	.63	.69	.48	.66	.80	.75	.85	XXX													
Tarsus length	.58	.55	.46	.55	.64	.63	.69	.71	XXX												
Rostrum length	.49	.47	.42	.43	.52	.54	.54	.50	.40	XXX											
Last rostral segment length	.18	.27	−.06	.17	.24	.23	.27	.28	.30	.14	XXX										
Antennal segment III length	.59	.58	.43	.63	.70	.66	.74	.74	.70	.46	.23	XXX									
Antennal segment IV length	.59	.57	.39	.54	.65	.63	.66	.66	.52	.37	.25	.56	XXX								
Antennal segment V length	.50	.62	.33	.52	.56	.52	.55	.60	.45	.29	.19	.58	.53	XXX							
Antennal segment VI length	.53	.59	.40	.57	.65	.63	.69	.67	.60	.41	.21	.69	.61	.62	XXX						
Sensoria number on segment III	−.04	−.11	.68	−.06	−.05	−.02	.03	.05	−.06	.12	.07	.12	.06	−.01	−.05	XXX					
Sensoria number on segment IV	.05	.01	−.07	−.03	−.03	−.10	−.01	−.06	.05	−.12	.11	−.02	.04	.03	−.04	.07	XXX				
Sensoria number on segment V	−.05	−.07	.60	−.12	−.06	−.04	−.05	−.05	−.14	.15	−.03	−.02	−.04	−.07	−.05	.15	.08	XXX			
Sensoria number on segment VI	.08	−.04	.04	.03	.01	−.02	.02	.00	.11	.04	−.11	.10	−.06	.04	.05	−.08	.30	.31	XXX		
Islands number on segment V	−.03	−.11	−.05	−.06	−.08	−.12	−.18	−.03	−.01	.07	.00	−.11	−.05	−.14	−.18	.24	.16	−.05	−.12	XXX	
Islands number on segment VI	−.04	−.11	−.03	.04	−.03	−.01	−.01	−.05	.08	.13	−.16	−.02	−.09	−.14	.28	−.02	.03	.12	.06	.00	XXX

Levels of significance: $P \leq .05 = r \geq .19$, $P \leq .01 = r \geq .24$.
All r values significant to $P \leq .01$ printed in light face roman.

few of type 3. It appears that the characters exhibiting intragall variance only do not show the influence of common intragall factors. The correlation coefficients of type 1 are rather high throughout and they are all positive. The length of the last segment of the rostrum, which is the only continuous character without intergall variance, shows poor correlation with the other characters, and head length and rostrum length, the other two poorly measured characters, show r values below the run of the table.

Three separate approaches are used to investigate which type 1 correlations are caused by common intergall factors and which few characters would best express these factors. These approaches are the analysis of covariance, the partition of the correlation coefficients, and factor analysis. The last is planned as a separate study in collaboration with Dr. Clyde P. Stroud.

The analysis of covariance

The first approach used was the analysis of covariance (for details see Snedecor, 1946, chapter 12). This analysis is a combination of the method of partitioning the variance of the dependent variate in regression with the method of analysis of variance. A significant F value for a covariance analysis of the regression A·B indicates that the unexplained variance of A, left after the explained variance of A due to variance of B has been accounted for, has a component of intergroup variance in addition to its intragroup variance. Translated into biological terms for this particular problem this has the following meaning. We are studying the relation of character A to character B. After the variance of character A due to variation of intergall and intragall factors affecting both A and B has been taken into account, we are left with a residual part of the total variance of A, the so-called unexplained variance. This variance is due to factors specific to character A. A significant value of F means that the specific factors are partly intergall factors, while a nonsignificant value for F means that so far as we know the entire unexplained variance of A is due to intragall factors.

Table 3 gives the results of the covariance analysis. Plus signs show the three levels of significance of F as before: +++ means $P \leq .001$, ++ shows $.001 < P \leq .01$, + shows $.01 < P \leq .05$, while

TABLE 3. *Levels of significance of F values for covariance*

Independent variable \ Dependent variable	Total length	Forewing length	Head length	Head width	Thorax length	Thorax width	Femur length	Tibia length	Tarsus length
Total length	(+++)	+++	———	++	+++	+++	+++	+++	++
Forewing length	+++	(+++)	———	+++	+++	+++	+++	+++	++
Head length	+++	+++	(++)	+++	+++	+++	+++	+++	+++
Head width	+++	+++	———	(+++)	+++	+++	+++	+++	++
Thorax length	+++	++	———	———	(+++)	++	+	++	+
Thorax width	+++	++	———	———	+++	(+++)	++	+++	+
Femur length	+++	++	———	+++	+++	+++	(+++)	———	———
Tibia length	+++	+++	+	+++	+++	+++	++	(+++)	———
Tarsus length	+++	+++	++	+++	+++	+++	+++	+++	(+++)
Rostrum length	+++	+++	++	+++	+++	+++	+++	+++	+++
Last rostral segment length	+++	+++	+++	+++	+++	+++	+++	+++	+++
Antennal segment III length	+++	+++	+	+++	+++	+++	+++	+++	———
Antennal segment IV length	+++	+++	+	+++	+++	+++	+++	+++	++
Antennal segment V length	+++	+++	++	+++	+++	+++	+++	+++	+++
Antennal segment VI length	+++	+++	+	+++	+++	+++	+++	+++	++
Sensoria no. on segment III	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sensoria no. on segment IV	+++	+++	++	+++	+++	+++	+++	+++	+++
Sensoria no. on segment V	+++	+++	++	+++	+++	+++	+++	+++	+++
Sensoria no. on segment VI	+++	+++	++	+++	+++	+++	+++	+++	+++
Islands no. on segment V	+++	+++	++	+++	+++	+++	+++	+++	+++
Islands no. on segment VI	+++	+++	++	+++	+++	+++	+++	+++	+++

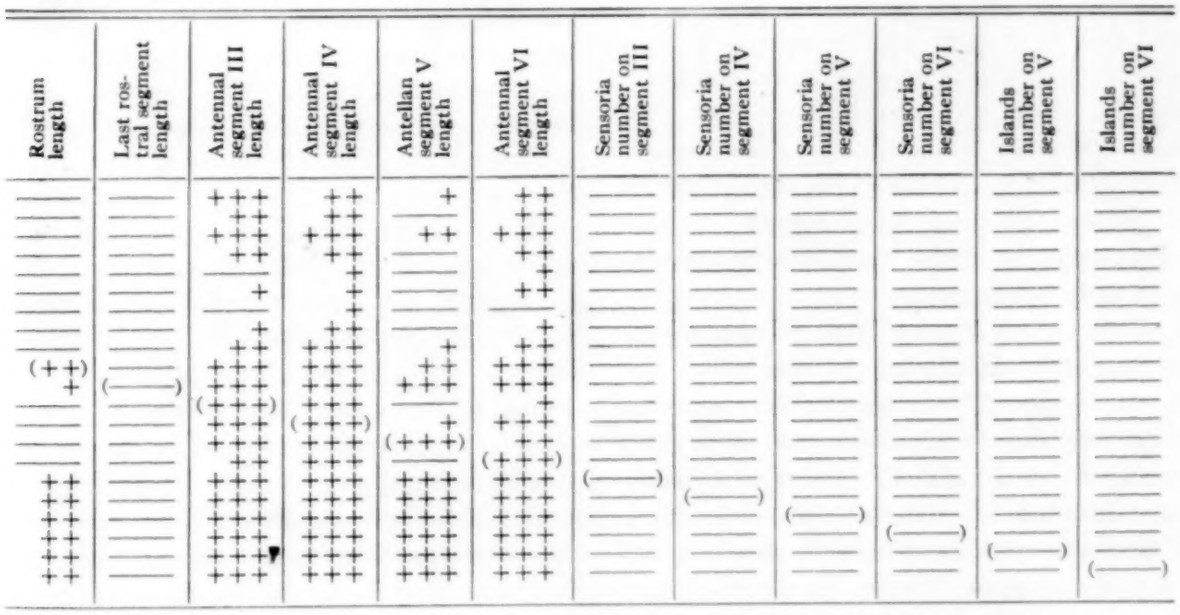
For explanation see text.

——— means F is not significant. The row of characters at the top of the table are dependent variates, i.e., the characters whose unexplained variance we want to test for intergall variance components when associated with the characters in the column at the left side of the table (the independent variates). For example, the three plus signs at the intersection of row 1 and column 2 mean that it is very likely that the unexplained variance of forewing length is at least in part due to intergall factors, when the wing length is studied associated with total length, and when common intergall and intragall factors affecting both forewing and total lengths have been accounted for. At the intersection points between identical characters, such as head length X head length there has been placed in parentheses the level of significance of the F value of the analysis of variance of that character.

An examination of table 3 reveals that the six meristic characters, as well as the length of the last segment of the rostrum have no intergall components in their unexplained variance regardless of which character they are associated with. This was to be expected since intergall differences did not enter into their total variance. On the other hand all characters having intergall variance components, when associated with the abovementioned seven characters, show intergall variance in their unexplained variance. This too was anticipated, since whatever intergall variance the continuous characters possessed could not be removed by association with the meristic characters and with length of the last rostral segment, that had no intergall variance themselves. Thus the intergall variance of the continuous characters was left in their residual, unexplained variance.

The previously discussed poor characters, head length and rostrum length, make a poor showing in table 3 as well. Most continuous characters remove their intergall variance, while they are not able to remove the intergall variance of most of these characters. Whether this is due to poor measurement or to the nature of the two characters is not clear. A third character, the length of antennal segment V, makes a poor showing. The same criteria hold for it. In any case, whatever intergall variance these three characters do possess is adequately accounted

analysis of measurements of alate Fundatrigeniae


Rostrum length
Last rostral segment length
Antennal segment III length
Antennal segment IV length
Antellan segment V length
Antennal segment VI length
Sensoria number on segment III
Sensoria number on segment IV
Sensoria number on segment V
Sensoria number on segment VI
Islands number on segment V
Islands number on segment VI

for by that of other characters, and thus head length, rostrum length and length of antennal segment V need not be used in a study of variation.

Turning to the good characters we first note thorax length. Other characters lose all or much of their intergall variance when associated with thorax length. Thorax length, on the other hand, maintains its intergall variance in its unexplained variance, whatever the character associated with it. Certainly, here is a very desirable character. Its intergall variance, presumably an expression of genetic differences, appears adequately to represent the intergall variances of most other characters. Yet none of these can represent the intergall variance of thorax length. If we wanted a single character to represent intergall variation in the population our choice would be thorax length.

Total length presents a different picture. Although no character can remove its intergall variance, yet all significant characters retain their intergall variance in their unexplained variances when in associations with total length. The explanation for this appears to be that, though it is like thorax length in being an expression of general length or size, total length is a composite measurement (thorax length, of course, is part of it) and has a greater variation due to this and the fact that position of the specimen on the slide and condition of the very soft abdomen influence it. This is reflected in the considerable coefficient of variation, 14.9, of total length. These circumstances make total length an undesirable character.

Although it appears that thorax length is a good expression of the most important intergall factor in this population, we note that there are important intergall residuals in the unexplained variance of a number of characters. These presumably represent different intergall trends of secondary importance, the variation of which might be very meaningful, however, over the geographic range.

The detailed reasoning which led to the choice of such characters is not given here, but can be surmised by the inspection of table 3. The basic criterion was retention of some intergall variance in the unexplained variance when in association with thorax length and of much intergall variance when in association with other characters. A further criterion was the ability of removing by association the intergall variance of as many characters other than thorax length as possible. In this manner forewing length, thorax width, femur length and antennal segment IV length were chosen.

Thus the original twenty-one characters were reduced to five characters which adequately represent the intergall variance of all the measured characters for this population. They presumably express any genetic variation present in the population. Some caution needs to be exercised in extending these results to the species population. We shall return to this point in the discussion.

The partition of the correlation coefficients

Another approach used in studying the relations between characters was the partitioning of correlation coefficients. The total correlations have already been reviewed (see table 2). We found all significant correlations to be either of type 1 or type 3. In the few cases of the latter type we have to assume common intragall environment, as the characters involved have no intergall variance. In the former case, however, correlation may be due to common intragall or intergall factors, or both, and it would be interesting to know how much, if at all, each class of factors contributed to the total correlation.

From the covariance analysis were obtained values for the intragroup sum of products and from the analysis of variance values for the intragroup sum of

squares. From these was derived a formula for the intragroup, in our case intragall, coefficient of correlation (see statistical appendix, note 3). This intragall correlation coefficient indicates the correlation of two characters when intergall factors are eliminated. It can thus be due to common intragall factors only, be these environmental factors within the gall or physiological factors that affect the two characters during development. The intragall correlations are shown in the upper half of table 4. They are generally quite low, the highest coefficient is .72, and significant correlations are all positive and distributed without apparent order through the matrix. Thus it appears that the major part of the correlations is due to intergall, and possibly genetic, factors.

Unfortunately it was impossible to construct a table of intergall correlations to match the intragall correlations. Such correlation coefficients would have to be based on the estimates of the true variance of means, σ^2_μ, mentioned previously in connection with the analysis of variance. Such estimates are not distributed normally, but rather follow a χ^2 distribution (see Crump, 1946). Thus a product moment formula for the correlation coefficient would be inapplicable. The author has thus far been unable to obtain a solution for this problem.

The next best procedure to follow is to use a correlation coefficient of means based on sum of products of means and sum of squares of means (see statistical appendix, note 4). Such a correlation coefficient expresses intergall correlation, but it is contaminated by intragall correlation since Σxy_m contains $1/k\Sigma xy_I$ and $\sigma^2_{X_m}$ and $\sigma^2_{Y_m}$ contain $1/k\sigma^2_{X_I}$ and $1/k\sigma^2_{Y_I}$ respectively. One solution to this problem would be to increase k, the number of individuals per group, until the contaminations became negligible. This is impractical in the present problem but would be applicable in cases where many individuals per clone are available for study. Even in our case, however, with $k = 4$, intragroup variance is sufficiently reduced to obtain a series of correlation coefficients that are at least indicative of the pattern of correlation of the true gall means.

The lower half of table 4 shows the correlation coefficients of gall means calculated in the manner described above. They are quite different from the intragall correlations and total correlations. There are no significant correlations of type 2 or 3, i.e., only those characters that possess intergall variance are significantly correlated, and all such type 1 correlations are significant. The r values are very high, ranging from .56 to .96, and are all positive. It might well be expected that, had it been possible to obtain correlation coefficients of true gall means, these would have been higher still. The significance of these correlation patterns will be discussed presently.

The Characters of the Stem Mother

The scarcity of measurable characters in the stem mothers has already been mentioned. Because only 25 fundatrices were available, the statistics derived from these measurements suffer somewhat due to size of their fiducial interval. A number of significant points emerge, however. In the two right hand columns of table 5 can be seen the means and coefficients of variation with their respective standard errors for the fundatrix characters. The coefficients of variation are of the same order or higher than those of the alates. This is presumably due to the skewness of the distributions which, although not as pronounced as in the alate measurements, was still considerable. The highest value of C.V. was 24 for total length. This is not only related to skewness but to the fact that total length is an extremely variable and seemingly unreliable character. It has been stated that the stem mothers are practically thin walled bags of embryos. Thus, when placed under a cover slip, some burst, others compress to varying degrees,

TABLE 4. *Intragall correlations and correlations of gall means between alate characters*

	Total length	Forewing length	Head length	Head width	Thorax length	Thorax width	Femur length	Tibia length	Tarsus length	Rostrum length	Last rostral segment length	Antennal segment III length	Antennal segment IV length	Antennal segment V length	Antennal segment VI length	Sensoria number on segment III	Sensoria number on segment IV	Sensoria number on segment V	Sensoria number on segment VI	Islands number on segment V	Islands number on segment VI
Total length	XXX	.23	.36	.50	.45	.60	.44	.35	.19	.35	.09	.29	.10	.24	.26	−.03	.14	.01	.13	−.07	−.07
Forewing length	.82	XXX	.01	.07	.38	.29	.21	.10	.03	.22	.19	.09	.14	.37	.26	−.14	.17	.05	.01	−.03	−.03
Head length	.84	.78	XXX	.17	−.02	.11	.18	.27	.36	.29	−.21	.22	.03	.08	.16	.19	−.06	.04	.06	.06	.06
Head width	.90	.85	.81	XXX	.26	.40	.17	.24	.15	.17	.10	.28	.02	.18	.29	−.06	−.04	−.07	.06	.08	.08
Thorax length	.86	.90	.81	.92	XXX	.72	.50	.45	.23	.42	.36	.30	.22	.15	.34	.07	−.01	.14	.09	.03	.03
Thorax width	.91	.89	.85	.95	.96	XXX	.49	.45	.25	.38	.26	.25	.22	.20	.41	.08	−.06	.05	−.10	.04	.04
Femur length	.80	.91	.74	.84	.94	.90	XXX	.58	.29	.44	.23	.41	.31	.21	.32	.16	.13	.04	−.18	.09	.09
Tibia length	.72	.91	.64	.82	.90	.85	.95	XXX	.39	.35	.19	.50	.38	.36	.35	.17	.02	.09	−.02	.06	.06
Tarsus length	.76	.81	.56	.76	.81	.80	.89	.88	XXX	.20	.19	.40	.13	.15	.32	−.05	.14	−.10	.03	.22	.12
Rostrum length	.66	.72	.60	.67	.68	.72	.68	.67	.62	XXX	.07	.37	.09	.03	.28	.23	−.10	−.23	.02	.02	.11
Last rostral segment length	.32	.43	.21	.30	.28	.30	.41	.46	.53	.30	XXX	.14	.19	.10	.18	.01	.13	−.04	−.14	.09	.09
Antennal segment III length	.71	.82	.62	.81	.86	.83	.89	.87	.89	.57	.42	XXX	.28	.29	.45	.22	.02	.09	−.15	.03	−.10
Antennal segment IV length	.80	.78	.71	.81	.83	.80	.83	.81	.78	.68	.38	.74	XXX	.35	.49	.07	.06	.07	−.09	.09	−.16
Antennal segment V length	.67	.80	.60	.76	.79	.72	.77	.78	.71	.63	.37	.81	.68	XXX	.41	−.03	.00	.04	.02	.02	−.10
Antennal segment VI length	.67	.77	.63	.72	.80	.74	.88	.85	.80	.56	.30	.84	.70	.80	XXX	−.06	−.05	.03	.04	.04	.02
Sensoria number on segment III	−.06	−.10	−.16	−.08	−.17	−.13	−.11	−.09	−.10	−.14	.26	−.08	−.12	.03	−.04	XXX	.08	.19	−.08	.31	.05
Sensoria number on segment IV	−.01	−.17	−.10	−.02	−.07	−.18	−.15	−.17	−.17	−.17	.05	−.08	.02	.07	−.02	.03	XXX	.14	.29	.19	−.06
Sensoria number on segment V	−.12	−.22	−.06	−.21	−.23	−.13	−.17	−.22	−.22	−.02	.00	−.17	−.21	−.27	−.17	−.01	−.11	XXX	.28	−.08	.24
Sensoria number on segment VI	.09	−.09	−.04	.00	.03	−.03	.07	−.03	.02	.09	−.03	.04	−.04	.08	.13	−.07	.34	.42	XXX	−.16	.06
Islands number on segment V	−.19	−.28	−.28	−.21	−.26	−.20	−.27	−.34	−.23	−.04	−.04	−.35	−.27	−.48	−.43	−.05	.04	.05	−.01	XXX	−.14
Islands number on segment VI	−.03	−.23	−.19	.00	−.08	−.06	−.09	−.18	.04	−.08	−.35	−.05	−.02	−.21	.05	−.25	.30	−.26	.05	.43	XXX

Intragall correlations above, correlations of gall means below diagonal.
Levels of significance: Intragall correlations—$P \leq .05 = \geq .22$, $P \leq .01 = r \geq .28$; Correlations of gall means—$P \leq .05 = r \geq .38$, $P \leq .01 = r \geq .49$.
All r values significant to $P \leq .01$ printed in light face roman.

while others are shrivelled up so that the lateral parts of the abdomen often are actually posterior to the cauda, or abdominal tip. Why this seemingly unsatisfactory character was included will emerge later.

The rest of table 5 shows the correlations of the fundatrix characters with themselves and certain alate characters. The first eight rows show the correlations among the stem mother characters themselves. We immediately note the generally low values compared to the r values of the total correlations of alate characters in table 2. Also there are very few significant ($P \leq .01$) coefficients. Head width appears most correlated with other characters, but even those r values are not impressive. Thus the measured fundatrix characters do not appear as closely knit together as their alate counterparts.

The subsequent five rows give the correlations between the fundatrix characters and the five alate characters chosen by the covariance analysis. The correlations are between gall means of the alate characters and the value of the character for the stem mother of that gall. When first calculated, fundatrix total

TABLE 5. *Measurement statistics and correlations of stem mother characters*

	Head width	Femur length	Tibia length	Tarsus length	Antennal segment III length	Antennal segment IV length	Last rostral segment length	Total length	$\bar{X} \pm \sigma_{\bar{X}}$	C.V. $\pm \sigma_{C.V.}$
Head width	xxx	.51	*.43*	*.39*	.61	*.35*	.73	*.46*	.454 ±.0061	6.7± .95
Femur length		xxx	.59	*.27*	.51	*.33*	*.25*	*.18*	.410 ±.0075	9.1±1.29
Tibia length			xxx	*.29*	*.36*	*.24*	*.39*	*−.18*	.337 ±.0071	10.5±1.49
Tarsus length				xxx	*.22*	*−.01*	*.31*	*.18*	.0925±.00213	11.5±1.63
Antennal segment III length					xxx	.55	*.45*	*.34*	.151 ±.0032	10.5±1.49
Antennal segment IV length						xxx	*.16*	*.12*	.1087±.00203	9.3±1.32
Last rostral segment length							xxx	*.33*	.0866±.00270	15.6±2.26
Total length								xxx	2.23 ±.105	24 ±3.5
Alate Characters										
Forewing length	*.42*	*.22*	*−.22*	*.13*	*.41*	*.28*	*.19*	.78		
Thorax length	*.39*	*.26*	*−.17*	*.11*	*.32*	*.24*	*.22*	.75		
Thorax width	*.34*	*.13*	*−.27*	*.14*	*.25*	*.12*	*.20*	.77		
Femur length	*.40*	*.25*	*−.17*	*.24*	*.39*	*.19*	*.26*	.79		
Antennal segment IV length	*.37*	*.33*	*−.09*	*.24*	*.44*	*.21*	*.06*	.63		
Head width	*.30*	—	—	—	—	—	—	—		
Tibia length	—	—	*−.11*	—	—	—	—	—		
Tarsus length	—	—	—	*.29*	—	—	—	—		
Last rostral segment length	—	—	—	—	—	—	*.22*	—		
Antennal segment III length	—	—	—	—	*.22*	—	—	—		
Antennal segment VI length	—	—	—	—	—	*.37*	—	—		
Total length	—	—	—	—	—	—	—	.61		

All linear measurements are in mm.
Levels of significance for correlations: $P \leq .05 = r \geq .40$, $P \leq .01 = r \geq .51$.
All r values significant to $P \leq .01$ printed in Light Face Roman.

length was not included and the writer was astonished by the complete absence of any significant ($P \leq .01$) correlations between stem mothers and alates which after all are isogenic. The writer had hoped for large correlation coefficients in order to be able to use stem mothers and their progeny interchangeably in his study of geographical variation. As an afterthought the total length of the stem mothers was measured and when correlated with the alate characters showed relatively high, significant correlations throughout, in spite of the tremendous variation of the stem mother character. This peculiar phenomenon is discussed in the next section.

Finally it was thought that perhaps the correlations between stem mother characters and their alate homologues might be higher. These r values are given in the last seven rows of table 5 for those fundatrix characters whose homologues are not included in the five chosen alate characters. Except for the correlation between the two total lengths the values again were not significant.

Discussion

The choice of characters

The main problem of this paper has been the choice of a set of characters representative of genetic variation within the species population. Such characters should describe the several major trends or factors determining the expression of all the characters studied or, if only one major trend is present, then they should describe this and some of the minor trends accounting for the residual variance of the characters. The validity of the covariance analysis method rests on the basic assumption that intergall variance is at least in part due to genetic differences from gall to gall. This assumption cannot be proven conclusively with the material at hand, but a number of *a priori* reasons have been given for the contention. There is additional circumstantial evidence to be obtained from the subsequent analyses.

The high correlations were shown to be largely because of high intergall correlations. If these are not due to common genetic factors, they must be due to common intergall environmental factors. The fact that the meristic characters and the last segment of rostrum length, all with intragall variance only, showed low significant correlation among themselves and in combination with other characters would indicate that the influence of a general intragall environmental factor was negligible. Again the intragall correlations of the characters with intergall variance were low and spotty, lacking a general intragall environmental factor. If such a general environmental factor is absent within galls, is there any particular reason for assuming its existence between galls?

The main trend emerging is an intergall general size factor (further discussion below). Where such factors are known in mammals and birds these have been identified as genetic (see Wright, 1932). There appears no reason to assume a different causation in insects. The argument may be advanced that insects vary much more in size than vertebrates and that such variations are frequently the result of differences in nutrition (see Mayr 1942, p. 29). An inbred, genetically homogeneous strain of *Drosophila* produces an impressive range of body sizes, apparently caused by nutritional variation. If then the size differences from gall to gall are nutritional effects, we should expect such a general influence on an intragall basis as well, since it is inconceivable that the hundreds of nymphs and alates crowding the inside of the gall, probably competing for space and certainly disturbing each other, should have identical nutrition. Yet intragall variance for most characters is quite low.

Finally, gross morphological intergall differences in the alates have been observed by the author. The lot studied

in this paper contained one gall with all the alates showing what appears to be a deleterious mutation—an inhibition of antennal differentiation showing the various degrees of penetrance common to phenomena of this sort from slight inhibition of differentiation to complete loss of the four distal segments. Thus there appear to be genetic differences in this population.

The characters of the stem mothers for this population, of course, cannot be evaluated through analysis of variance and covariance. The low correlations between the various characters are striking and might indicate lack of a major genetic trend and presence of a number of different trends or environmentally caused variation of these characters. It is interesting that these characters are not correlated with their homologues or other characters in the alates, and this might favor the view that they vary on a nongenetic basis. If that is so then the lack of environmentally caused correlations between fundatrix and alate characters could be explained by the differences in environment during the formative period of the stem mother and that of her offspring. The exception to the low correlations is total length of the stem mother, at face value a poor character, and yet this seems to be the only fundatrix character correlated with the major intergall trend.

An alternate interpretation of intergall variation, taking into account the fundatrix-alate correlation for total length, might be to ascribe the differences in general size to differences in the early nutrition of the stem mothers. I believe this to be unlikely since each stem mother at the time of collection had produced 200 alates by conservative estimate. For the nutritive influence to persist for so long it would have to affect the alates at an extremely early stage of embryonic development.

Assuming the variation of the characters chosen to be in part genetic, how legitimate is an extension of the results from the local population to the entire species population? No prognosis can be made on this point, but results of this nature are merely signposts toward the beginning of a variation study for an entire species. There will have to be numerous spot checks from various localities to see whether the present analyses of variance and the correlations hold for those populations. This is unlikely since Clark (1941) has shown geographic variation in correlation coefficients for measurements of *Peromyscus*.

Size factors

When a series of characters that are linear measurements of structures are studied, the question arises as to whether each structure is influenced by its own special size factor, be that genetic or environmental, or whether there is a general size factor influencing the size of all or most of the series. This question has been studied and debated for some time (see Wright, 1932). In an analysis of measurements of rabbits and hens, and of rabbit crosses, Wright (1932) came to the conclusion that a major part of the variance of the characters was due to differences in general size, while the remainder resulted from regional or group and special size factors. He also concluded that general size, and possibly other size factors, are initiated by genetic differences.

In this study the presence of an intergall general size factor is clear. Thorax length seems to express it best, but thorax width and total length are good indicators as well. It is significant that these three characters have a very low percentage of intragall variance (see table 1) and thus are relatively little influenced by intragall factors. From the high correlation of alate thorax length with fundatrix total length there is no doubt that the general size factor is present in the stem mothers as well and, in spite of the great accidental variance of this stem mother character, controls body size of

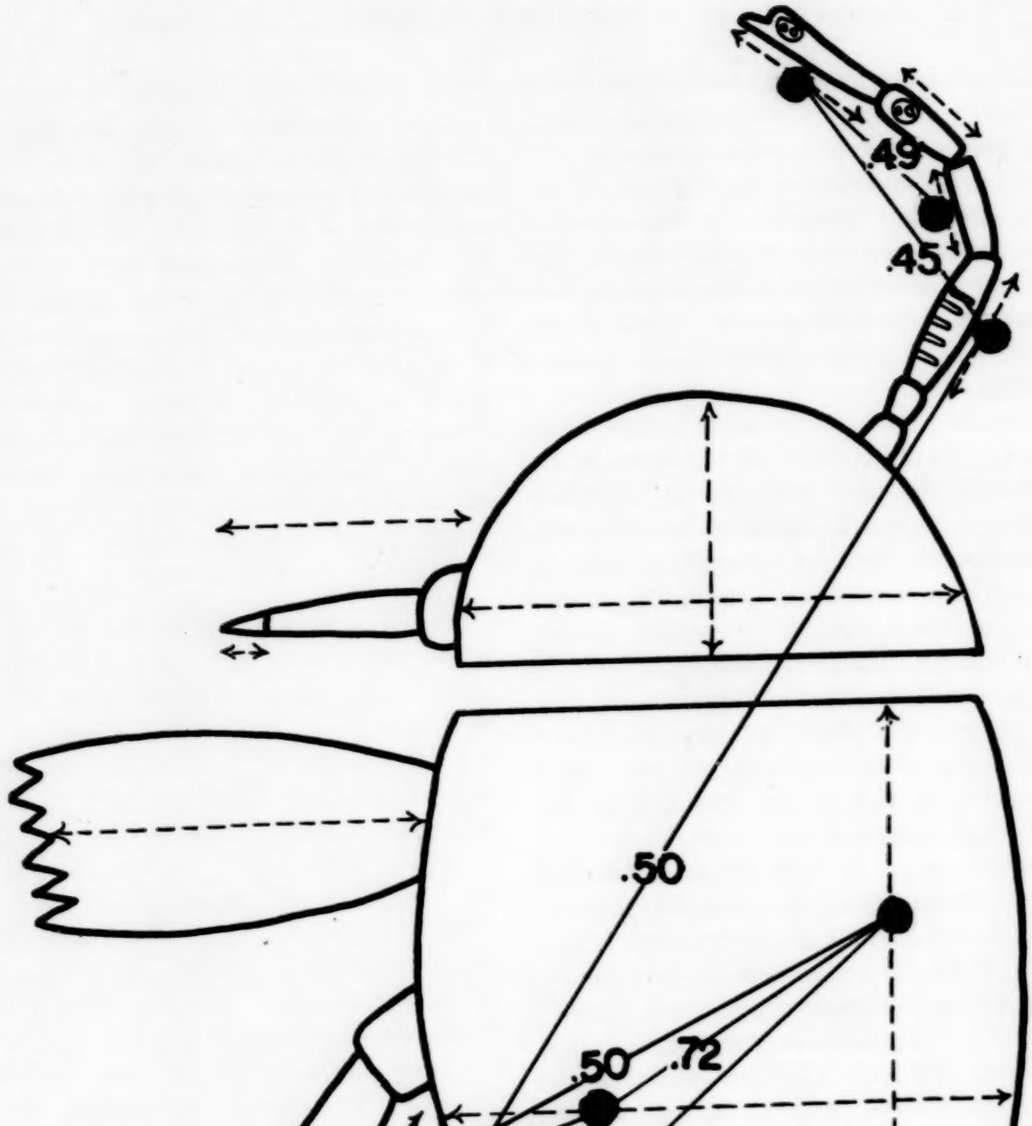


FIG. 1. Intragall correlations between alate characters. Correlations shown as solid lines, characters as broken lines. Only values of $r \wedge | .45$ have been drawn in. Level of significance of correlations: $P \leq .01 = r \geq .28$.

the fundatrices rather well. It is peculiar, however, that none of the other stem mother characters are significantly influenced by it.

The presence of group factors cannot be decisively demonstrated without factor analysis or path coefficients, but there are indications of such to be gleaned from the covariance table (table 3). For example, tibia length and tarsus length both have some residual intergall variance when associated with thorax length. When they are associated with femur length this variance disappears.

For special size factors there is abundant evidence. The matrix of intragall correlation coefficients (table 4, upper half) shows some medium sized correlations and these would indicate regional, non-hereditary, physiological influences. Chase (1939) studied the correlations between various areas on the coats of guinea pigs for frequency of color spots. The strains used were inbred; thus genetic differences were ruled out. He found correlations between adjacent areas indicating regional non-hereditary factors.

When an arbitrary distance code was given to each pair of alate characters correlated, the largest code to those characters farthest apart, the lowest to those closest together, and when this code was correlated with the intragall r values, a significant ($P \leq .01$) value of $r = -.28$ was obtained, indicating that there is a slight negative correlation of distance between two characters and their intragall correlation coefficient. Figure 1 shows all intragall correlations of .45 or more. All of them except tibia length X length of antennal segment III are between structures close to one another. This would indicate the presence of some local, non-genetic influences contributing to the final expressions of the characters.

Finally, the possession of a large correlation matrix makes it possible to investigate the application of the so-called rules of correlation to *Pemphigus*. We shall only mention Pearson's rule that adjacent organs are more highly correlated than those farther apart. Alpatov and Boschko-Stepanenko (1928) studied the antennae of the hemipteran *Pyrrhocoris apterus* and found adjacent antennal segments more highly correlated than alternate ones and these higher than the correlation between segments I and IV. The correlations decreased as one progressed distad, perhaps due to lessened control by the factors affecting the body.

In *Pemphigus* relations are not so simple. The pattern of differentiation of the antennae is as follows: VI off III, then V off III, then IV off III. The total correlations bear this out. The highest r value (.69) is between III and VI, then .58 between III and V and .56 between III and IV. VI is only insignificantly more highly correlated with V (.62) than with IV (.61). IV is closer to VI (.61) than to V (.53). When the other significant continuous characters are correlated with antennal lengths we find the mean of their r values with III to be .62, with VI to be .57, while the expected order with IV and V is reversed, .55 and .49 respectively. In the stem mothers all characters show consistently higher correlations with length of antennal segment III than with length of segment IV. Thus Pearson's rule holds generally with modifications for order of differentiation. A study of leg correlations confirms this conclusion for both alates and fundatrices.

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Summary

1. A local population of the gallmaking aphid *Pemphigus populi-transversus* was studied with a view to finding characters suitable for a study of intraspecific variation.

2. Twenty-eight galls, representing that number of clones, were used and the stem mother and four alates of each gall were measured. Twenty-one characters were measured for each alate and eight for each stem mother.

3. Means and coefficients of variation are given for each character measured. The values of the latter are relatively high due to anormality of the data.

4. By analysis of variance, one continuous and six meristic alate characters were shown to lack intergall variance and thus owe their variation to intragall, environmental factors only. The remaining alate characters exhibit significant variance between galls.

5. An analysis of covariance demonstrated that the intergall variance of thorax length accounts for much of the intergall variances of other alate characters. Thus thorax length was found to be the most representative alate character.

6. Other characters were chosen on the basis of their relative independence from thorax length. Forewing length, thorax width, femur length and antennal segment IV length were so chosen.

7. Total correlation coefficients were calculated and showed a lack of correlation between the non-significant meristic characters and other characters as well as high correlations among the significant characters.

8. The total correlation coefficients were partitioned into intragall and intergall correlation coefficients. There are low non-hereditary intragall correlations between characters, while the intergall correlation are high for all significant characters.

9. Stem mother characters showed low correlations among themselves and with alate characters, except for stem mother total length which is well correlated with alate characters.

10. The assumption that intergall variance is in part due to genetic factors is made and defended.

11. The presence of a general size factor of probably genetic origin is believed demonstrated for the first time in insects. There is some indication of group size factors.

12. Non-genetic special size factors are shown for some characters and there is some evidence that intragall correlations are a function of distance between correlated characters.

13. Pearson's rule on correlations can be shown in modified form in antennal and leg correlations.

Statistical Appendix

1. $\sigma^2_\mu = \frac{1}{k}(\sigma^2_{GR} - \sigma^2_I)$. Since variance between groups

$$\sigma^2_{GR} = \frac{k}{n-1}\sum^{n}(m_X - \overline{X})^2$$

$$= k\sigma^2_m = \sigma^2_I + k\sigma^2_\mu.$$

2. Percentage of variance determined by intragall factors $= \frac{\sigma^2_I}{\sigma^2_\mu + \sigma^2_I} \times 100.$

3. Intragroup coefficient of correlation,

$$r_{xy_I} = \frac{\Sigma xy_I}{\sqrt{\Sigma x^2_I \Sigma y^2_I}}.$$

From sum of products within groups,

$$\Sigma xy_I = \sum^{n}\sum^{k}(X - m_X)(Y - m_Y),$$

and sum of squares within groups,

$$\Sigma x^2_I = \sum^{n}\sum^{k}(X - m_X)^2$$

and

$$\Sigma y^2_I = \sum^{n}\sum^{k}(Y - m_Y)^2.$$

4. Correlation coefficient of means,

$$r_{xy_m} = \frac{\Sigma xy_m}{\sqrt{\Sigma x^2_m \Sigma y^2_m}}.$$

From sum of products of means,

$$\Sigma xy_m = \frac{1}{k}\Sigma xy_{GR}$$

$$= \overset{n}{\Sigma}(m_X - \overline{X})(m_Y - \overline{Y}),$$

and sum of squares of means,

$$\Sigma x^2{}_m = \frac{1}{k}\Sigma x^2{}_{GR} = \overset{n}{\Sigma}(m_X - \overline{X})^2,$$

$$\Sigma y^2{}_m = \frac{1}{k}\Sigma y^2{}_{GR} = \overset{n}{\Sigma}(m_Y - \overline{Y})^2.$$

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HETEROPLOIDY IN MOUSE EMBRYOS DUE TO CROSSING OF INBRED STRAINS

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Introduction

The diploid chromosome number characteristic of the house mouse (*Mus musculus*) is 40, but spontaneously heteroploid eggs or early embryos can occur, as reported in earlier papers (Beatty and Fischberg, 1949 and 1951a; Fischberg and Beatty, 1950 and 1951). These eggs with abnormal chromosome numbers were found mainly in a few strains of mice which carried the recessive mutation "silver" (*si*). Triploidy was the most frequent type, but other types such as haploidy, tetraploidy, hexaploidy, aneuploidy, multiform aneuploidy and diplo-polyploid mosaicism were also encountered. In a preliminary paper (Fischberg and Beatty, 1950) we considered the factors responsible for this spontaneous heteroploidy. The most striking fact was that silver females mated to males of other strains not carrying the mutation silver gave significantly more eggs with abnormal chromosome numbers than any other combination, and more even than the combination silver by silver. Table 1 gives the numbers and percentages. We suggested that the high proportion of heteroploid eggs might be due to a) a general tendency towards heteroploidy in silver strains, and also b) crossing of different strains, involving perhaps an incompatibility between the eggs of silver females and the sperm of non-silver males.

The present work was undertaken to answer the following questions: is the high incidence of heteroploid eggs in the cross of silver females to males of other strains characteristic only for these silver females, or is it true in general that crossing of different mouse strains raises the proportion of heteroploid eggs? Should the latter be generally true, then the high incidence of heteroploidy in certain types of silver matings would be seen in a wider perspective, and it would be of interest to examine the possible role of crossing between different strains in the evolution of chromosome numbers.

Material and Methods

Planning of the experiments. The three well-known inbred strains A, C57 and CBA were used. All three have been inbred brother to sister for many generations. The individuals in any one of these inbred strains are much alike, and are a relatively constant factor in the experiment. The experiment consisted of two parts, which were run, in the main, concurrently:

1) Control matings (= within strain matings).
2) Cross matings (= between strain matings).

As controls we took females mated with males of the same inbred strain, and counted the chromosomes of their eggs or early embryos. This was done for all three strains. In the cross matings, each female was mated with a male of a different strain. The reciprocal crosses were also made. There were thus six different combinations in the cross mating experiment.

The primary intention of the experiment was to compare two main types of mating, viz. *within inbred strains* with

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TABLE 1. *Spontaneous heteroploidy in 3½-day embryos from different mating combinations of silver and control mice.* From Fischberg and Beatty, 1950. Control mice do not carry the mutation silver (si). Silver mice are always homozygous for si. Experiment 3 has since been greatly enlarged and the percentage of heteroploid embryos has not dropped.

Experimental series	Mouse strain used ♀ × ♂	No. of ♀♀ with analyzable embryos	No. of ♀♀ with one or more heteroploid embryos	♀♀ containing one or more heteroploid embryos as percentage of total ♀♀ with analyzable embryos	Total number of analyzable embryos	No. of heteroploid embryos	Heteroploid embryos as percentage of total analyzable embryos
1	control × control	50	1	2.0	173	1	0.6
2	silver × silver	53	7	13.2	206	8	3.9
3	silver × control	58	17	29.3	269	25	9.3
4	control × silver	46	7	15.2	216	8	3.7

inbred strains crossed. The nine different combinations were used in order to give a better balance to the experiment, and to see whether there were any outstanding peculiarities of a specific combination. We did not expand the experiment to collect sufficient material to compare minor differences between each of the nine different combinations.

We planned to have for each of the 6 cross-matings at least 16 mice, each with one or more analyzable eggs, and from each of the 3 different within strain matings at least 32 animals, each with one or more analyzable eggs. Thus, the cross-matings and the within strain matings were each to contain a minimum of 96 mice. This figure was exceeded slightly.

Description of the three inbred strains. The sub-strains of the Institute of Animal Genetics were used—A/Fa, CBA/Fa and C57/Fa. Strain A/Fa is homozygous for the known mutations non-agouti (*a*), brown (*b*) and albino (*c*) (see Strong 1936a and Heston, 1949). Strain CBA/Fa is of wild-type appearance. CBA and A are related in origin (see Strong, 1936b, 1942, and Heston, 1949). Strain C57/Fa, unrelated to A and CBA, is homozygous for the mutation non-agouti (*a*) (see Little and Bagg, 1924, and Heston, 1951). Each strain has been inbred for many generations.

Recovery and preparation of eggs. As in earlier work, we thought it best to count chromosomes in the egg 3½ days after copulation. The eggs of this age are generally in the blastula stage and contain about 40–60 cells; the chromosomes are larger and more easily counted than in later stages. In what follows, we shall refer to these 3½-day-old eggs as *embryos,* in order to distinguish them from undivided eggs or early cleavage stages.

The embryos are recovered by washing out the uterine horns, and are stained with the modified Feulgen method of Slizynski (1949). This technique, and the method of cytological analysis, have already been described in Beatty and Fischberg (1951a). We did not attempt superovulation because there was a danger of introducing unknown factors which might influence the number of chromosomes.

RESULTS

Table 2 shows that nearly two-thirds of the numerous embryos recovered could not be analyzed, because they died before recovery, or were lost during fixation and staining, or contained no analyzable mitosis. In table 3 details of the 12 heteroploid eggs are presented.

The control results (within inbred strain matings). It may be seen from table 2 that when females of an inbred strain were mated with males of the same inbred strain, only 1 embryo out of a total of 327 was heteroploid. This pro-

TABLE 2. *Summary of mice used and embryos recovered*

	Grand total	Within inbred lines.				Inbred lines crossed						
		CBA × CBA	C57 × C57	A × A	Total	CBA ♀ × C57 ♂	C57 ♀ × CBA ♂	CBA ♀ × A ♂	A ♀ × CBA ♂	C57 ♀ × A ♂	A ♀ × C57 ♂	Tota
No. ♀ ♀ used	288	42	59	52	153	24	20	21	25	21	24	135
No. ♀ ♀ with embryos	251	39	50	46	135	19	18	18	22	19	20	116
No. ♀ ♀ with analyzable embryos	203	33	37	36	106	16	16	16	16	17	16	97
No. ♀ ♀ with heteroploid embryos	9	—	—	1	1	1	—	2	2	1	2	8
Average no. of embryos per ♀ with embryos	7.10	7.03	6.10	7.65	6.90	7.74	7.28	7.17	7.41	7.05	7.35	7.34
Total no. of embryos	1782	274	305	352	931	147	131	129	163	134	147	851
Unanalyzable, dead and lost embryos	1129	182	208	214	604	106	76	76	98	84	85	525
Total analyzable embryos	653	92	97	138	327	41	55	53	65	50	62	326
Diploid embryos	641	92	97	137	326	40	55	50	62	49	59	315
Triploid embryos	6	—	—	1*	1*	1	—	1	1	—	2	5
Tetraploid embryos	1	—	—	—	—	—	—	—	—	1	—	1
Aneuploid embryos	3	—	—	—	—	—	—	2	1	—	—	3
Mosaic embryos	2	—	—	—	—	—	—	—	1	—	1	2
Total heteroploid embryos	12	—	—	1*	1*	1	—	3	3	1	3	11

* This single triploid embryo in the A×A matings is the same as given in the controls of table 1. It has been taken over to table 2, together with a few diploid embryos from other mice whose matings fulfilled the conditions for the present work.

portion (0.3% of the total) is negligible. The fact that heteroploidy does not occur spontaneously (or is negligible) if mice of the same strain are mated together is further supported if the 173 control embryos of table 1 (series 1) are also considered: of these, 35 diploid and 1 triploid embryo were taken over into table 2 because the embryos were derived from matings which fulfilled exactly the conditions of the present experiment. The remaining 137 embryos (all diploid) came from mice which were mated with males of the same strains, though they were not

TABLE 3. *Protocol of the heteroploid embryos.* Embryos were recovered 3½ days after copulation. Most chromosome counts were made on metaphases. The ± sign means that chromosomes were counted with a general order of accuracy of ±5% of the number of chromosomes, or better, but a number following the ± sign indicates the range within which the true count certainly lay. The + sign alone means that a few extra chromosomes were probably also present, but could not be counted.

Inbred line of parents	Serial No. of ♀	Serial No. of embryo	Chromosome numbers of heteroploid embryos	Assessment of type of heteroploidy
A ♀ × A ♂	47	c	57±; 61±	triploid
CBA ♀ × C57 ♂	1	c	60±	triploid
CBA ♀ × A ♂	13	e	53±; 57±; 60±; 53±	triploid
CBA ♀ × A ♂	19	c	43; ca. 39	aneuploid
CBA ♀ × A ♂	19	d	44	aneuploid
A ♀ × CBA ♂	6	a	47±; 50±; 40+	aneuploid, hyperdiploid
A ♀ × CBA ♂	6	e	60±2	triploid
A ♀ × CBA ♂	15	d	39±; 40±; 79±; ca. 100	diplo-heteroploid mosaic
C57 ♀ × A ♂	5	e	80	tetraploid
A ♀ × C57 ♂	19	a	59±1	triploid
A ♀ × C57 ♂	19	b	40±; 60+	diplo-heteroploid mosaic
A ♀ × C57 ♂	24	a	58±; 54+; 52+	triploid

inbred strains in the formal sense. If these 137 diploid embryos are added to the number in table 2, then we have only 1 heteroploid embryo out of a total of 464. Similar results from still another control experiment could be added. To summarize: *if mice of the same strain are mated together, no heteroploid embryos result, or only a negligible number.*

The cross mating results (inbred strains crossed). The females of inbred strains mated with males of other inbred strains produced 11 heteroploid embryos (3.4%) out of a total of 326, or, in terms of mice, 8 females out of a total of 97 (8.2%) produced one or more heteroploid embryos each (table 2). This incidence of heteroploidy is not negligible. The actual percentage of heteroploidy may be still higher, as probably an unduly high proportion of the unanalyzable embryos (see table 2) are likely to be heteroploid, for reasons which have been discussed in Beatty and Fischberg, 1951a. The conclusion drawn from this experiment is that *crossing of different inbred strains* of Mus musculus *produces heteroploid embryos.*

Comparison of control and cross mating results. The comparisons below are made on the basis of the experiments planned for the present work. It will be recalled, however, that we have accessory data from other work showing that within strain matings give an even lower yield of heteroploid eggs than reported here. The significance of the differences calculated below would be even greater if these accessory data were taken into account. Table 4 summarizes the number of analyzable embryos given in table 2, and table 5 lists in the same way mice with analyzable embryos. As indicated before, the small numbers make no detailed comparison possible between the single combinations and we compared therefore only the total results of within strain matings with the total results of crosses between inbred strains. If we take the number of heteroploid embryos (1) and of diploid embryos (326) in the within strain matings and compare them with the heteroploid embryos (11) and diploid embryos (315) produced in the cross matings, then the adjusted $\chi^2 = 6.90$, whence $P = 0.009$. The proportion of heteroploid embryos is therefore significantly higher in the cross matings than in the controls.

We have now to investigate if the proportion of *mice* bearing heteroploid embryos is also significantly higher in the cross matings than in the controls. The numbers for mice in the controls are: 1 mouse with 1 heteroploid embryo against 105 mice with only diploid embryos. In the cross matings there are 8 mice with one or more heteroploid embryos, against

TABLE 4. *Summary of numbers of diploid and heteroploid embryos recovered from the different mating combinations.*

♂ \ ♀	CBA		C57		A	
	heteroploid embryos	diploid embryos	heteroploid embryos	diploid embryos	heteroploid bryos	diploid embryos
CBA	0	92	0	55	3	62
C57	1	40	0	97	3	59
A	3	50	1	49	1	137

TABLE 5. *Summary of numbers of mice with only diploid embryos, and of mice which contained one or more heteroploid embryos, grouped after the different mating combinations.*

♂ \ ♀	CBA		C57		A	
	with heteroploid embryos	with only diploid embryos	with heteroploid embryos	with only diploid embryos	with heteroploid embryos	with only diploid embryos
CBA	0	33	0	16	2	14
C57	1	15	0	37	2	14
A	2	14	1	16	1	35

89 mice with only diploid embryos. The adjusted χ^2 testing independence of classification between the controls and the cross matings series is 4.77, which gives a P of 0.03. If Fisher's exact method is used, a P value of 0.0249 results: this figure is the value obtained by Fisher's method (0.01243), multiplied by 2 to obtain a P value comparable with the P resulting from the χ^2 test. Thus it seems reasonable to regard the proportion of mice bearing heteroploid embryos as significantly higher in the cross matings than in the controls. It may be mentioned again that if accessory data from other work were included, the degree of significance would be increased.

The conclusion that heteroploidy occurs more often if mice of different inbred strains are crossed than if mice are mated within the same inbred strain, seems to be generally valid for all mating combinations used, except for the mating of C57 females with CBA males (tables 4 and 5), but the absence of heteroploid eggs in this particular mating may well be a consequence of the small numbers used rather than a significant departure from the general rule.

General Discussion

The different types of heteroploidy. Table 3 shows that the commonest type of heteroploidy in the 12 heteroploid eggs is the triploid (6). Other types are the tetraploid (1), the aneuploid (3), and the diplo-heteroploid (2). The relative proportion of the different types is of the same order as that reported in other work (Beatty and Fischberg, 1951a; Fischberg and Beatty, 1950); for further details of the different types of heteroploid eggs see also Beatty and Fischberg, 1951b and 1952, and Fischberg, and Beatty, 1951.

The mechanism of origin of the heteroploid embryos. How can we explain the different results from matings within strains compared with matings between strains? Triploidy most probably arises between the time of the entrance of the sperm into the egg and the time of fusion of the pronuclei. An earlier or a later origin is almost certainly excluded in all experiments so far performed by the authors. As against an earlier origin is the fact that it is of importance what kind of sperm is concerned. This is evident in the present work, and was also reported earlier, in that silver females mated with males of other strains gave a significantly higher number of heteroploid embryos than other combinations (Fischberg and Beatty, 1950). We have further been able to show that a hot shock applied at the time of the entrance of the sperm produces a high proportion of triploidy (Fischberg and Beatty, 1952). As against an origin of triploidy *after* fusion of the two haploid pronuclei is simply the difficulty of conceiving any possible mechanism which could lead from a diploid zygote nucleus to a triploid one; as far as we know, no mechanism of this kind, leading regularly to triploidy, has been reported in any organism.

There are two main ways in which an embryo can become triploid; the egg provides two sets of chromosomes and the sperm only one; or the sperm (or sperms) provide the two sets. It is not yet possible to decide if one or the other or both kinds of origin occur. But it seems more likely that the egg provides the two sets, and the sperm only one, as shown by temperature shock induced triploidy in newts—Fischberg, 1948 (genetically), and Fankhauser and Godwin, 1948 (cytologically). Further, at the time of fertilization, the sperm has already reduced its chromosome number to the haploid set, and there is no evident way in which it could contribute two chromosome sets to the egg—except by polyspermy, and we know of no evidence from mammals that polyspermy contributes extra chromosome sets to the egg. The egg, on the other hand, is still diploid up to the moment of fertilization, and needs the stimulation of the sperm to get rid of the second chromosome set by extrusion of a polar body; if this polar

body should fail to form, then the egg could contribute two chromosome sets.

The tetraploid type almost certainly has its origin during, or shortly after, the *first cleavage division* of the egg (see Beatty and Fischberg, 1952), by failure of anaphase movement of the daughter chromosomes, or by a fusion of the daughter chromosomes or dividing daughter nuclei into a single tetraploid mitosis which will eventually give a tetraploid resting nucleus. A mechanism of this kind occurs in newts as well (Sanada, 1951; Fischberg, unpublished data).

Aneuploid embryos arise after abnormal meiotic or cleavage divisions. The time of their origin is difficult to trace. The same mechanism can be responsible for the production of mosaic embryos.

The greater frequency of heteroploidy in the cross of silver females with non-silver males, as compared with other combinations. We mentioned earlier that certain mouse strains which are homozygous for the mutation silver (*si*) produce a number of heteroploid, mostly triploid, embryos. If silver females are mated with males of other strains (non-silver) a considerably higher proportion of heteroploid embryos is produced. How can these observations be explained?

In eggs of silver mice, presumably the maturation divisions and the early cleavage divisions do not always proceed normally. Something in the mechanism of division of certain eggs does not function as satisfactorily as in other strains of mice. We do not know what the reasons for these abnormalities are. We know, however, that mammalian eggs, including those of the mouse, are arrested at the stage of metaphase or early anaphase of the second maturation division. The eggs wait for the sperm, whose entrance stimulates the process of anaphase movement of the egg chromosomes and finally leads to the expulsion of the second polar body, which contains one set of maternal chromosomes. Observations in many forms of animal show how delicately balanced and complicated these processes of fertilization, maturation and cell division are (see also Pesonen, 1946, 1949; Vara and Pesonen, 1947).

The experiments described in this paper indicate that the entrance of a sperm of one inbred strain into the egg of another inbred strain may disturb the delicate balance of these processes of fertilization, maturation and cell cleavage and can therefore lead to heteroploidy. This suggests that differences, which may be very slight, exist between the sperms of different strains and also between the eggs of different strains. These differences are perhaps of a chemical nature.

A rather different kind of explanation of the higher yield of heteroploid embryos after crossing of strains needs discussion. This explanation invokes a heterosis effect, in that a highly heterozygous triploid egg may have a greater viability than a less heterozygous one; the latter may well die before examination. We have however not adopted this explanation because of the hot shock experiments, which often involved germ cells of the same strain, and yet the high proportion of triploid embryos survived.

The foregoing now leads to a *two-factorial explanation* of the high incidence of heteroploidy in crosses of silver females with males of other strains. Matings of silver females with silver males yield 3.9% heteroploid embryos. This suggests that in silver mice the processes of maturation (females only?) and probably early cleavage divisions are not as well balanced as in other strains, which produced only 0.3% of heteroploidy. If silver males are mated with females of other strains we get 3.7% of heteroploid embryos. This percentage is however similar to the 3.4% which has been observed in the present cross matings with inbred lines and seems due entirely to crossing of two different strains. If silver females are mated with males of other strains, then 9.3% heteroploid embryos can be found (table 1). This high

incidence of heteroploidy can be explained by combination of the two factors. First, the processes of maturation and probably early cleavage division in silver eggs are not as well balanced as in the eggs of other mice; and in addition these processes are disturbed by the entrance of sperm which is foreign to the strain.

If the percentages of heteroploid embryos which occur as a consequence of the two factors described are added, then we would expect to find about 7.3% heteroploid embryos (3.4% from crossing of different strains and 3.9% from silver by silver matings). We observed in fact 9.3% heteroploid embryos when silver females were mated with males of other strains. The difference between 7.3% and 9.3% is not significant. Simple addition of the two percentages thus gives a figure which is not significantly different from the observed figure for silver mice. On the other hand, simple addition is a rather rough-and-ready method, and a more complicated relation is not excluded. For instance, the processes of nuclear and cell division in silver eggs may be more sensitive to the entrance of a foreign sperm than in non-silver eggs.

Although we cannot prove whether two sets of chromosomes are brought in by the egg or the sperm in triploid eggs, we favor the first possibility, which would be analogous to most experimental results with Amphibia. If the second possibility were true, then our explanation of the high incidence of heteroploid eggs in silver mice would have to be modified to allow for a role of polyspermy instead of an effect on the processes of maturation and early cleavage of the egg.

Possible evolutionary consequences of the occurrence of heteroploidy due to crossing of strains. Near-related species often differ in their chromosome numbers. The difference may range from one single chromosome in the haploid set to differences equivalent in number to one or more haploid sets. It is known that species of *Drosophila* can vary by only one or two chromosomes. Svärdson (1945) suggested that recent Swedish Salmonoids are old polyploid species, and that a few of the existing species differ from each other by 10 chromosomes, or a number which is near to 10 in the haploid set. Svärdson thinks 10 may represent the basic number for Salmonoids. Kupka (1948) demonstrated that four different forms of Swiss Salmonoids (*Coregonus*) have diploid complements of 36, 70, 72 and 72 chromosomes. It is not clear so far if these forms of *Coregonus* are true species or sub-species. In the *Muridae* (after Matthey 1949) the following diploid chromosome numbers are found: *Rattus* 42 (4 species), 46 (2 species); *Mus* 40 (5 species); *Apodemus* 46 (1 species), 48 (7 species), 50 (1 species); *Nesokia nemorivaga* 46; *Micromys minutus* 68; *Arvicola scherman* 36; *Microtus* 28, 32, 46, 50, 56 (different species); *Eothenomys melanogaster* 56; *Evotomys* 56 (2 species); *Sigmodon hispidus* 54; *Neotoma floridana* 52; *Cricetus auratus* 28; *Cavia cobaya* 64; *Geomys breviceps* 84; *Perognathus fallax* 44; and *Dipodomys merriami* 86. Some of the higher numbers are either exactly or nearly twice as large, or half as large again, as lower numbers and there is perhaps a suspicion of a polyploid relation; though, as Matthey stresses, it is also important to take into account the number of chromosome arms.

The main theories concerning this variation of the chromosome number in a group of animals are: fragmentation of one, several or all chromosomes, non-disjunction during maturation divisions, fusion of chromosomes, and polyploidy as a consequence of temperature shocks and other mechanisms.

In the light of the present results on crossing of inbred strains of *Mus musculus,* it is suggestive to compare these inbred strains with small, strongly isolated populations. This comparison will, of course, be of value only if further experiments should demonstrate that the pro-

duction of heteroploidy after crossing of different strains or populations is a general phenomenon, and not valid only for domesticated mice. Inbreeding by brother × sister matings leads quickly towards a uniform genetic constitution of all the individuals of the same line. A similar, though less rapid, trend exists in any small, isolated population in nature. If for some reason the isolating barriers between two or more small isolated populations break down and allow a mating between the individuals of the different populations, then heteroploidy (mainly triploidy) may occur frequently. If some of these heteroploid individuals can survive, become fertile, and mate with diploid or heteroploid individuals, then a high variation of chromosome number and genotype would be created. One or the other of these new types may show a positive selection value for the specific qualities of the environment. This would then be the basis for a new sub-species, which in time might become a true species.

We are of course aware of the many factors which may militate against the establishment of new chromosome numbers. But new chromosome numbers certainly have arisen during evolution. It is further known that many plant species can survive as polyploids and can be fertile; the same holds for some animals, e.g., *Ambystoma mexicanum* (Humphrey and Fankhauser, 1949). *Triturus pyrrhogaster* and *Rana nigromaculata* (Kawamura, 1951a, b). A great variation of chromosome number and genotypes arises when fertile triploid individuals reproduce after mating with diploids (Fankhouser and Humphrey, 1950; Kawamura, 1951a, b). Variation of this kind would be expected to play a role in evolution.

The present results suggest that the crossing of parents even of the same chromosome number leads to heteroploidy in some of the progeny, provided that the parents are of different strains.

Conclusions

Chromosomes were counted in 3½-day-old embryos recovered from females of three inbred strains of *Mus musculus*, mated in nine different ways. Matings within a strain resulted in a negligible number of heteroploid embryos (0.3%). Matings between strains gave a significantly higher percentage (3.4%), the commonest type of heteroploidy being triploidy. It is concluded from the present results, in conjunction with previous work with "silver" strains of mice, that crossing of different strains of mice raises the proportion of heteroploid embryos. In silver matings there seems to be a separate factor increasing the incidence of heteroploidy still further. The mechanism of origin of heteroploid embryos is discussed. An analogy is made between the crossing of inbred strains of mice and the crossing of small isolated populations in nature due to break-down of the isolating barriers. Possible evolutionary consequences are discussed.

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INTESTINAL YEAST FLORAS OF SUCCESSIVE POPULATIONS OF DROSOPHILA

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Introduction

In 1948, Dobzhansky expressed the opinion that yeasts are important in understanding some of the forces of natural selection to which the natural populations of *Drosophila* are exposed. This opinion was based on preliminary experiments which showed that different yeasts effected population changes differently. Information concerning the occurrence of yeasts in *Drosophila*, however, is sparse, a few isolates being made by Dobzhansky and Epling (1944), Wagner (1944 and 1949), Buzzati-Traverso (1950) and Hedrick and Burke (1950).

The need for information on the distribution and occurrence of yeasts in these flies and on plants in the various regions during population changes is apparent. The writers therefore have pursued a study with the view of obtaining information on (1) the relation between yeast flora and cyclic changes in *Drosophila* populations, (2) the possible location of breeding places of *Drosophila* by isolation of yeasts from various plants in a specific area.

Experimental Procedures

Collection of flies

Collections were made in the Pinon Flats and Keen Camp areas on Mt. San Jacinto in Southern California. These localities were chosen because Dobzhansky and his colleagues had been collecting flies there for a number of years. Furthermore, these areas vary in elevation and vegetation, thereby offering an opportunity to consider yeast ecology.

Fermented banana baits in cups were used for trapping the flies. In order to prevent the flies from feeding on baits, the fermented banana (at the bottom of the cup) was covered with a piece of paper towel above which was placed a wire mesh. Flies were removed from these traps every 10 to 15 minutes by covering the cup with a funnel leading to a test tube in which the flies were finally gathered. Collections were made in the evenings about one hour before sunset.

Transportation of flies

The flies were cooled to about 0° C. immediately after collection and then shipped at this temperature to Berkeley, California, by air express, in accordance with the procedure outlined by Shihata and Mrak (1951). The flies were shipped the evening of collection and platings for yeasts were made the next morning.

Dissection of flies and isolation of yeasts

In order to obtain the intestinal yeast flora, it was necessary to modify certain techniques. Excretory deposits could not be used since practically all the yeasts cells in them were dead (Shihata and Mrak, 1951). Dissection of the fly was essential, but the following procedures described in the literature proved unsatisfactory because of the danger of contamination: (1) removing the head and the tip of the abdomen, and gradually withdrawing the alimentary tract; (2) triturating the insects in a suitable medium; (3) pulling the head and at-

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tach digestive tract (or at least crop) from the insect.

The procedure found to be most satisfactory was a modification of the conventional method (Balfour-Browne 1932) for insects. Immediately after killing in ether, flies were partially embedded in paraffin wax, dorsal side up. Sterilized miniature scalpels made from flexible razor blades, and very fine needles made from No. 0 pins were the instruments used for dissection. A longitudinal slit was made along each of the lateral lines through the whole thorax and abdomen so the dorsal integuments could be pulled away. Then the head and tip of the abdomen were cut, after which the entire alimentary canal was removed from its surroundings for plating. Each alimentary tract was streaked on propionate-wort-agar (Mrak and Phaff and Douglas, 1948) with the view of obtaining yeast growth with a minimum of mold growth. The plates were incubated at 20° C. for 7 days during which period frequent examinations and isolations (as new yeasts appeared) were made. Purification of the isolates was achieved by making at least three successive transfers from single colonies.

Yeasts from other sources such as plant materials were obtained by direct streaking and by soaking the material in sterile water for several hours before streaking a drop of the water.

Identification of yeasts

With certain exceptions the procedures of Stelling-Dekker (1931), Lodder (1934), and Diddens and Lodder (1942) were used to obtain morphological and physiological characteristics used for classification. Durham rather than Einhorn fermentation tubes were used because of the greater sensitivity of the former (Bouthilet *et al.*, 1949). Melibiose prepared according to the procedure of Skinner and Bouthilet (1947) was used, rather than raffinose, in van Iterson-Kluyver tubes, when there was any indication of raffinose fermentation in Durham tubes. In addition to the auxanogram media of Lodder, those recently formulated by Wickerham (1946) and Wickerham and Burton (1948) were also employed.

The vegetable extract-agar medium of Mrak *et al.* (1942) (with yeast added) as well as those of Stelling-Dekker was used for sporulation. The presence or absence of carotinoid pigments was determined by the procedure of Mrak *et al.* (1949). Alcohol utilization was determined in the medium of Wickerham and Burton (1948) as well as that used by the Dutch workers.

Yeasts Isolated from Drosophila Flies and Other Sources Collected in the Pinon Flats Region

Yeasts from D. pseudoobscura

Collections of flies were made in 1950 on March 30, April 16 and 24, and May 12. The first collection was made later than normal because of the occurrence of cold weather until late in March. Collection was discontinued in May because of the scarcity of flies as a result of hot weather. Nevertheless, collections for this investigation coincided with the seasonal changes of flies as observed by Epling and Mitchell (1950). Twenty flies from each of the first three collections and eight from the last were dissected and plated for yeast although all did not yield yeast. The results of the yeast isolations are summarized in table I.

The comparatively few yeast colonies growing on plates from collections made on March 30 and April 16 is attributed to the use of a thermos bottle filled with crushed ice for cooling during transportation. When a "super-ice" package was used in place of the thermos bottle (Shihata and Mrak, 1951) a greater number of colonies was observed (see results for April 24 and May 12 in table I). Digestion of the yeasts by the flies was

TABLE I. *Yeasts occurring in D. pseudoobscura flies collected at Pinon Flats during the spring of 1950*

Taxonomic designation of isolate	Number of flies yielding particular organism	Total number of colonies of particular organism	Date of sample collection
Saccharomyces fragilis	5	8	3/30
Torulopsis pulcherrima	4	11	3/30
Torulopsis bacillaris	2	2	3/30
Candida krusei	2	5	3/30
Rhodotorula glutinis	2	2	3/30
Candida parapsilosis	1	4	3/30
Candida guilliermondii	1	2	3/30
**Pichia mrakii*	1	6	3/30
Torulopsis colliculosa	1	1	3/30
**Zygosaccharomyces drosophilae*	1	1	3/30
Candida lipolytica	2	2	4/16
Candida parapsilosis	1	1	4/16
**Zygosaccharomyces drosophilae*	1	11	4/16
Pichia bispora	1	1	4/16
Saccharomyces cerevisiae	13	1624	4/24
Candida krusei	1	100	4/24
Torulopsis fermentans	1	1	4/24
Oospora lactis	1	1	4/24
Kloeckera lindneri	3	504	5/12
**Zygosaccharomyces drosophilae*	2	64	5/12
Zygosaccharomyces marxianus	1	100	5/12
**Zygosaccharomyces mrakii*	1	23	5/12
Candida krusei	1	5	5/12
Saccharomyces fragilis	1	5	5/12

* A new species will be described in a separate publication.

much slower when held at a lower temperature during shipping.

It is of interest that only one or two species of yeasts were isolated from a single fly. It is also of interest that the yeast flora of each collection was relatively undiversified and composed of but few genera. The significance of these observations will be considered in the discussion.

In March, the yeast flora was composed mostly of *Saccharomyces fragilis* and species of *Torulopsis* and *Candida*. In April, the flora consisted mostly of *S. cerevisiae*. In May, the yeast flora was composed largely of *Kloeckera lindneri* and three species of *Zygosaccharomyces*. These data indicate that the types of yeast ingested by *D. pseudoobscura* may vary from time to time.

Yeasts from soils and plant materials

Although it is known that certain slime fluxes serve as breeding places for flies (Carson, 1951) there is reason to believe that there must be other places. With the hope of obtaining information that would aid in locating additional feeding places of the flies, yeasts were isolated from soil and plant materials collected in the Pinon Flats area. The results are summarized in table II.

S. cerevisiae and *C. parapsilosis*, isolated respectively from Juniper berries and soil collected April 16, were also isolated from flies. When these collections were made, many larvae were found in Juniper berries but none were identified as *Drosophila*.

On April 24, only *R. glutinis* was isolated from Juniper and Manzanita ber-

TABLE II. *Yeast isolated from soil and plant materials at Pinon Flats during the spring of 1950*

Taxonomic designation of isolate	Approximate number of colonies of particular organism per plate	Source of isolation	Date of sample collection
Kloeckeraspora apiculatus	300	Juniper berries	4/16/51
Kloeckeraspora apiculatus	1	Juniper berries	4/16/51
Torulopsis aeria	100	Exudates of pine trees	4/16/51
Pullularia pullulans	30	Cactus stem	4/16/51
Pullularia pullulans	20	Cactus stem	4/16/51
Pullularia pullulans	30	Soil	4/16/51
Pullularia pullulans	2	Exudates of pine tree	4/16/51
**Torulopsis granulosa* var. *nitida*	10	Cactus stem	4/16/51
**Torulopsis granulosa* var. *nitida*	6	Cactus stem	4/16/51
Saccharomyces cerevisiae	10	Juniper berries	4/16/51
Candida parapsilosis	1	Soil	4/16/51
Rhodotorula glutinis	400	Manzanita berries	4/24/51
Rhodotorula glutinis	400	Juniper berries	4/24/51
Pichia chodati var. *trumpi*	330	Cactus stem	5/12/51
Torulopsis albida	300	Cactus stem	5/12/51
Torulopsis albida	10	Exudate of pine tree	5/12/51
Torulopsis rotundata	170	Yucca and rhizome	5/12/51
Rhodotorula glutinis var. *rubescens*	73	Exudate of pine tree	5/12/51
Pullularia pullulans	3	Cactus stem	5/12/51
Pullularia pullulans	2	Yucca and rhizome	5/12/51

* Will be described in a separate publication, new variety.

ries. This is in contrast to cultures of *Kloeckeraspora apiculatus* and *S. cerevisiae* from Juniper berries collected on April 16. Yeasts were not obtained from soil and tree exudate samples collected on April 24.

On May 12, Juniper berries were no longer available for sampling. The yeasts obtained on this date were different from those isolated from previous collections, except in the case of *P. pullulans*.

The yeasts isolated from *D. pseudoobscura* were distinctly different from

TABLE III. *Yeasts occurring in D. pseudoobscura flies collected at Keen Camp during the spring of 1950*

Taxonomic designation of isolate	Number of flies yielding particular organism	Total number of colonies of particular organism	Date of sample collection
Saccharomyces cerevisiae	1	405	4/24/51
**Zygosaccharomyces drosophilae*	1	10	4/24/51
Kloeckera lindneri	1	1	4/24/51
Oospora lactis	1	1	4/24/51
**Zygosaccharomyces drosophilae*	10	1084	5/21/51
Zygosaccharomyces pastori	5	58	5/21/51
Zygosaccharomyces marxianus	2	3	5/21/51
Kloeckera lindneri	6	307	5/21/51
Torulopsis aeria	1	12	5/21/51
Candida krusei var. *van laeriana*	1	1	5/21/51
Pullularia pullulans	1	1	5/21/51

* Will be described in a separate publication, new species.

those isolated from soil and plants in the Pinon Flats area, and in only two cases were similar organisms found.

Yeasts Isolated from Flies and Other Sources in the Keen Camp Region

Yeasts from D. pseudoobscura

Three collections of flies were made on April 16 and 24 and May 21. Although twenty flies from the first collection were dissected no yeasts were isolated. Most of the plates remained sterile, although a few showed some bacterial and fungal growth. This was the direct result of inadequate cooling after collection and during shipment. Isolations from the yeast flora obtained from *D. pseudoobscura* collected at Keen Camp changed quantitatively as well as qualitatively from one month to another; see table III.

Yeasts isolated from plant materials and insects

Material was collected on April 24 from every source suspected as a possible breeding place for *D. pseudoobscura* in the Keen Camp area. No yeasts were isolated from pine tree exudates or soil. The types and occurrence of yeasts isolated from sources given in table IV were not consistent with those obtained from flies. However, two species of yeasts, also occurring in flies, namely, *Kloeckera lindneri* and *Z. marxianus*, isolated from bark beetle larvae and leaves of a shrub infested with a lepidopterous insect respectively, were collected at Keen Camp (see table IV).

Table IV. *Yeasts isolated from plants and other materials at Keen Camp at different times*

Taxonomic designation of isolate	No. of colonies of particular organism per plate	Source of isolation
**Candida pseudoalbicans*	400	Moist wood of dead pine tree
**Candida pseudoalbicans*	400	Moist wood of dead pine tree
**Candida pseudoalbicans*	400	Moist wood of dead pine tree
Candida catenulata	100	Wood from fallen tree infected with insects
Candida parapsilosis	17	Moist bark of injured pine tree
Torulopsis rugosa var. *fermentans*	300	Flower of cactus
Torulopsis pulcherrima var. *variabilis*	30	Moist wood of dead pine tree
Torulopsis gropengiesserii	3	Flower of a weed
Torulopsis rotundata	1	Flower of wild onion
Torulopsis rugosa	1	Flower of wild onion
Pichia chodati var. *trumpi*	300	Moist bark of injured pine tree
Pichia belgica	100	Moist bark of injured pine tree
Pichia belgica	30	Moist bark of injured pine tree
Pichia belgica	3	Moist bark of injured pine tree
Zygosaccharomyces fermentati	150	Moist bark of injured pine tree
Zygosaccharomyces fermentati	1	Moist bark of injured pine tree
Zygosaccharomyces marxianus	1	Leaves from a shrub infected with lepidopterous insect
Pullularia pullulans	56	Leaves from a shrub infected with lepidopterous insect
Pullularia pullulans	10	Bark beetle
Kloeckera lindneri	4	Bark beetle larva

* New species, will be described in a separate publication.

Discussion

Dobzhansky (1943) showed that the population of *D. pseudoobscura* inhabiting Pinon Flats exhibits cyclic changes

which are due to natural selection. Changes in population of *D. pseudoobscura* were also observed at Keen Camp by Dobzhansky (1943) and Epling and Mitchell (1950). The yeast flora obtained from flies in two areas also varied with date of collection.

The variation of yeast flora may affect the populations of the flies in different ways. Different yeasts have different fermentative and ester production characteristics (Guilliermond, 1920, and Stelling-Dekker, 1931) and hence attractiveness for the flies. Barrows (1907) showed that various esters differ in their capacity to attract *Drosophila ampelophila*. Buzzati-Traverso (1950) found that different yeasts varied in their power to attract *Drosophila*. Another supporting experiment was carried out by da Cunha *et al.* (1950) who found that different yeasts isolated from two different species of *Drosophila,* attracted the flies differently. Therefore, it is probable that one effect of yeasts on the populations of *Drosophila* is the result of a difference in fermentation characteristics of different yeasts.

Another effect of yeasts on the populations of *Drosophila* may be due to variations in composition. There are significant variations in the vitamin composition of different species and strains (see, for example, Burkholder, 1943; Burkholder and Moyer, 1943; Rogosa, 1943; and Christophersen and Holzweissig, 1950). Wagner (1944 and 1949) found that two species of *Drosophila* of the *mulleri* group differed physiologically because they varied in ability to grow and develop on media inoculated with various yeasts. Consequently, it appears that various types of the flies differ in their growth requirements. Since from time to time yeasts in a given area may vary, the growth and development of one type of fly may be favored at one time and some other type at another time.

Aside from the observations of da Cunha *et al.*, 1950, there is no conclusive experimental evidence to show that flies of different gene arrangements may differ in their ability to grow and develop on different yeasts. The data collected, however, has established the importance of pursuing further studies in this area.

It has been pointed out that a single fly never yielded more than three species of yeasts. In most instances only one species of yeast, and occasionally two, were isolated. This indicates the presence of specific yeast in predominating numbers in the feeding substrate at the time of feeding, and perhaps a choice on the part of the fly. The number of yeast available to the fly and its opportunity for some choice offer a possible explanation for the month to month variation in the intestinal flora of the flies.

The breeding places of *Drosophila* at Pinon Flats and Keen Camp are unknown. Comparing yeasts isolated from the flies to those isolated from various other sources in these localities, one finds that only twelve species out of thirty-three were obtained from both flies and other sources. Although the new species, *Zygosaccharomyces drosophilae,* was isolated from the flies collected in the localities mentioned above, it was not isolated from other sources in these areas.

The breeding places remain unknown except for the observation of Carson (1951), although the results of this investigation indicate that further consideration should be given to the possibility of some sort of an association with local trees. The exudates of various trees merit intensive study, since Carson has found the larvae of certain species of *Drosophila* in these substances at Mather, California and at St. Louis, Missouri.

Summary

(1) Yeasts were isolated from *Drosophila* flies collected at Pinon Flats and Keen Camp, and from other sources in these areas. The flies were trapped by use of fermented protected banana baits, then transferred to test tubes which in turn placed in a "super-ice" package

and shipped to Berkeley by the fastest means of transportation available.

(2) Yeasts isolated from the flies collected at Pinon Flats and Keen Camp varied from month to month.

(3) Yeast flora of flies collected at any one time from any of the localities, previously mentioned, was homogeneous. Usually one species of yeast and occasionally two or three, were isolated from a single fly. *Drosophila* inhabiting the different localities in the early summer showed a similarity in the occurrence of species of *Zygosaccharomyces*.

(4) The isolation of yeasts from sources other than flies did not lead to the detection of the breeding places of flies, but it did emphasize the need for further studies on the yeast flora from trees (particularly pine and oak) in the particular areas.

Acknowledgments

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THE ESTIMATION OF ADAPTIVE VALUES OF EXPERIMENTAL POPULATIONS[1]

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INTRODUCTION

The concept of adaptive value is one of the fundamental concepts of population genetics. The adaptive value of the carriers of a given genotype is measured by their contribution to the gene pool of the next generation. Carriers of different genotypes within a population and also different populations of a species may be compared as to their adaptive values.

The adaptive value is a quantity that can be measured more or less easily depending on the nature of the material under study. Periodic determinations of the frequency of an easily recognized mutant gene or a chromosomal aberration in a population under constant environmental conditions yield reliable information about the adaptive values of carriers of this gene or aberration. The adaptive values are estimated from the rates of change in frequency and the equilibrium frequencies of the various genotypes. (See Wright and Dobzhansky, 1946, for typical experiments and calculations.)

The estimation of adaptive values is more difficult for genetic factors that are not easily identifiable, or for a complex that can be broken up by recombination. Such difficulties are encountered if two chromosomes from different localities but with similar gene arrangements are to be compared, or if individuals carrying the genetic complex typical of population A are to be compared with corresponding individuals from population B. In these cases estimates of adaptive values must be made by studying physiological factors known to be concerned in reproduction and perpetuation of populations—viability, longevity, fertility, sexual activity, and so forth. These studies may not yield accurate estimates of the adaptive values, for several reasons: (1) The estimates arrived at through a comparison of different physiological traits are often different: sterile flies, for example, are often fully viable. (2) The adaptive value is an outcome of the interaction of numerous physiological traits, whereas only the most obvious ones can be subjected to study. (3) The experimental conditions always differ from the environmental conditions of the populations —even of experimental populations. Despite the dubious accuracy of estimates of adaptive value arrived at in this fashion, such estimates may be useful in understanding the nature of the selective forces that operate in populations. The relative "well-being" of populations may be inferred on the basis of such estimates.

In the course of studies of experimental populations of *Drosophila melanogaster* subjected to irradiation(Wallace and King, 1951; Wallace, 1951), various attempts were made to determine the relative adaptive values of these populations. The results of these studies are reported in this paper.

MATERIAL AND METHOD

The histories of the experimental populations are summarized in table 1; more detailed accounts are given in the papers cited above. In short, several fairly similar populations of *D. melanogaster* were kept for a number of generations under a variety of conditions of irradiation; and some of the physiological properties of these populations were then compared.

[1] This work was done under Contract Number AT-(30–1)-557, United States Atomic Energy Commission.

TABLE 1. *Short histories of the five experimental populations*

Population	Approx. size (No. adults)	Starting date	First sample number	Irradiation
1	10,000	7/25/1949	1	Original ♂♂-7000 r X-ray Original ♀♀-1000 r X-ray
3	10,000	7/25/1949	1	none
5	1,000–	4/1/1950	20	2000 r/gen., chronic, gamma
6	10,000	4/15/1950	21	2000 r/gen., chronic, gamma
7	10,000	4/15/1950	21	300 r/gen., chronic, gamma

Since the comparisons were intended to reveal the *genetic* effects of irradiation on populations, precautions were taken to avoid studying individuals suffering from the debilitating *somatic* effects of direct irradiation. The flies used in these studies either were obtained from egg samples removed from the sources of radiation or were offspring of flies that hatched from these eggs.

The tests were carried out at 25° C.: detailed procedures of the different experiments will be given with the data.

GENETIC TESTS

The estimation of the adaptive value of the carriers of various genotypes was based on the average frequencies of wild-type flies appearing in the F_3 cultures of the matings shown in figure 1. Wild males (P_1) obtained from population egg samples were mated individually to *Cy L/Pm* females. *Cy*, *L*, and *Pm* designate the three dominant genes Curly (wing), Lobed (eye), and Plum (eye color); the *Cy L* chromosome also carries two inversions that suppress crossing over. Single F_1 *Cy L/ +* males were taken from the P_1 cultures and were backcrossed to *Cy L/Pm* females. Since each *Cy L/ +* male carried but one wild second chromosome, all the F_2 *Cy L/ +* flies in each culture carried identical wild second chromosomes. By systematically intercrossing *Cy L/ +* males and females from the offspring of different fathers, F_3 cultures were obtained that produced *Cy L/ +* and wild-type flies in approximately a 2:1 ratio. The wild-type flies in each culture were identical with respect to their second-chromosome constitution; each carried two second chromosomes obtained from two different P_1 males. The frequency of wild flies in each F_3 culture, then, was a measure of the viability of individuals carrying those two chromosomes; in different cultures the viability varied from lethal to "normal" (approximately 33% wild flies). A series of tests from a number of populations permitted a comparison of the relative viabilities of the individuals from the different populations.

The estimates of adaptive values obtained for each population by this technique were remarkably uniform. In a series extending over more than 20 generations (or, more precisely, "two-week sample intervals") in the populations listed in table 1, only population 3 had average frequencies of wild flies that seemingly varied with time; the frequencies in this population increased at an average rate of 0.1% per generation (the error of this average was 0.04%, and the probability that this increase was the result of chance fluctuations was .02). Because the values were practically constant, summarized data are given in table 2, together with the adaptive values indicated by the data. At the time of the first tests, populations 5 and 6 had been subjected to about 14,000 r of gamma radiation and population 7 to about 2,000 r; at the time of the last tests included in the data, 5 and 6 had received some 60,000 r and 7 about 9,000. Populations 1 and 3 had existed for 28 generations at the time of the first tests, and over 50 at the time of the last ones. The genetic variability induced by X-radiation in

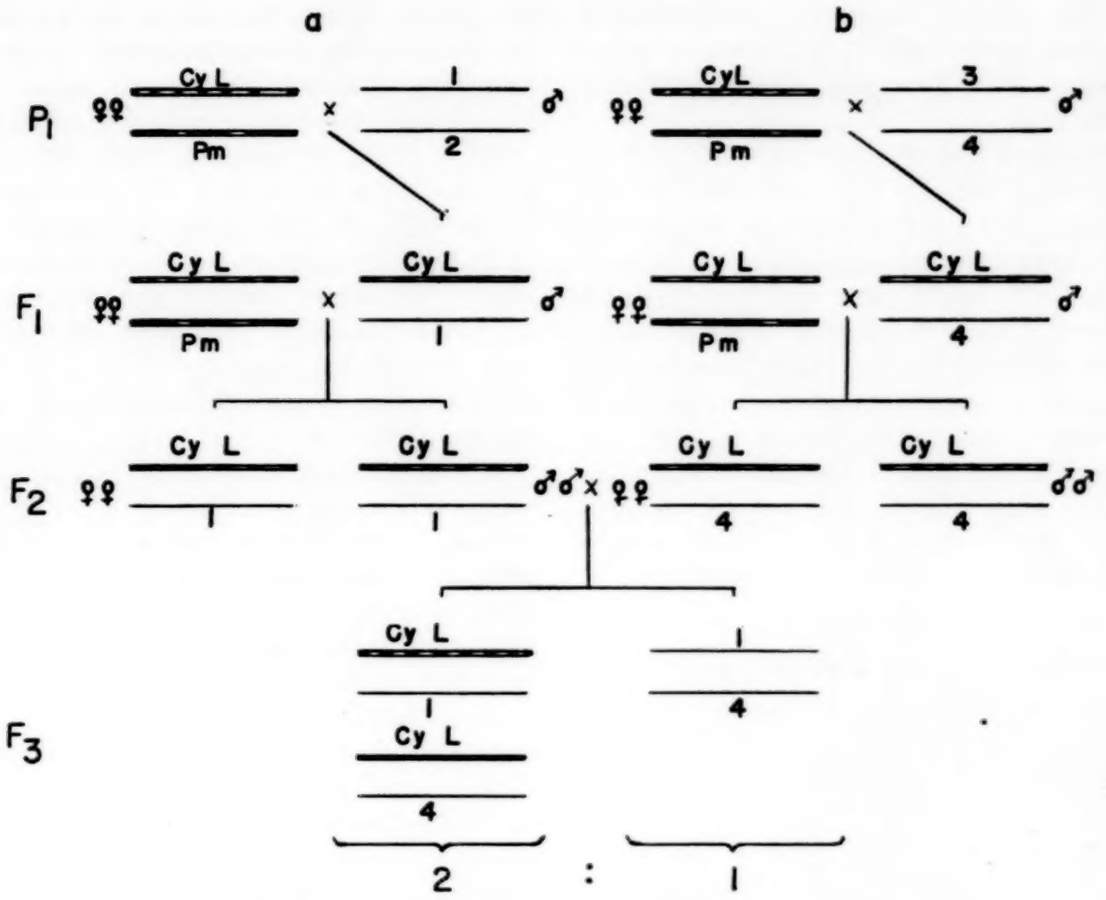

FIG. 1. The genetic test for estimating the adaptive values of populations.

population 1 had therefore been exposed to a great deal of natural selection. The data in table 2 show that the populations differed in adaptive value, and that those receiving chronic irradiation apparently had lower adaptive values. The differences noted are probably significant except that, as indicated above, population 3 showed an improvement and was approximately equal to population 1 in the later samples. The number of flies counted to establish these values was approximately one million.

TABLE 2. *Average frequency of wild flies in F_2 cultures of the routine test described in the text*

Population	Cultures tested	Per cent wild-type	Adaptive values
1	1454	35.06	1.03
3	1181	34.14	1
5	1007	31.85	.93
6	1326	32.48	.95
7	1350	33.40	.98

MALE FERTILITY

For all genetic tests of the second chromosome, wild males obtained from egg samples were mated singly with *Cy L/Pm* females. Since only a single F_1 *Cy L/+* male was wanted from each of these crosses, the matings were made in vials. The procedure in making these matings was fairly constant: the P_1 males were picked from the sample bottles within 14 days after the date of the sample, and were stored for 2–5 days in vials; the *Cy*

TABLE 3. *Estimations of adaptive values based on the frequency of fertile P_1 males from the different populations*

Population	Total cultures	Sterile cultures	Per cent fertile	Adaptive values
1	5299	181	96.58	1.01
3	5324	225	95.77	1
5	4816	722	85.01	.89
6	6171	836	86.45	.90
7	5866	227	96.13	1.00

L/Pm virgins were raised in heavily yeasted bottles and aged 2–7 days before mating; 2 or 3 virgins were mated with each male.

Some of the P_1 cultures produced no offspring, because the male died or was sterile. A record of these sterile matings was kept after the initiation of populations 5, 6, and 7; the data are given in table 3. Since frequency of fertile males must be related to the adaptive value of a population, estimates of this value could be calculated from the data, and are shown in the table. The males of population 7 were slightly but not significantly more fertile than those of 3, the control population.

Longevity

Three longevity experiments were made, in which flies from two or three of the five populations were studied simultaneously for comparison. Somewhat different procedures were used in each of these experiments; a standard technique to be used routinely in experiments of this type has not yet been established. In two of the experiments (a and b below) a certain number of flies was placed in each of a series of half-pint bottles, and the survivors were counted each week. Under these conditions, the life span of the survivors is influenced by the number of deaths that have occurred because longevity depends to some extent on population density (Pearl and Parker, 1922). This relationship interferes with the use of the variance of the mortality as a measure of phenotypic variance—especially if the patterns of early mortality differ. A test of isolated flies was made (c below) but this presented difficulties that limited the number of flies in the experiment. Detailed accounts of the studies follow.

a. Populations 6 and 7. Mass subcultures hatching from sample 31 of populations 6 and 7 were the source of the flies of this first experiment. Adult males and females not more than 24 hours old were collected daily and placed in half-pint cultures with yeasted cornmeal-agar-molasses food. Except in two cases, the number of flies placed in each culture was 100 (50 males and 50 females); in those two cases the number was 70. Each week the survivors were etherized, counted, and placed in fresh culture bottles. At the time of this experiment population 6 had received an aggregate dose of 20,000 r; population 7, 3,000 r. The frequency of lethal second chromosomes was approximately 40% in population 6 and 10% in population 7.

Table 4. *Survival of flies from populations 6 and 7 after ten generations of irradiation.* % = per cent surviving; $\mathring{x}$ = mean longevity (days); a.v. = estimated adaptive value

	Population 6				Population 7			
Weeks	males	%	females	%	males	%	females	%
0	250	100	322	100	448	100	523	100
1	233	93.2	307	95.3	442	98.7	513	98.1
2	213	85.2	291	90.4	415	92.6	487	93.1
3	196	78.4	274	85.1	388	86.6	451	86.2
4	184	73.6	239	74.2	359	80.1	406	77.6
5	160	64.0	174	54.0	296	66.1	273	52.2
6	112	44.8	82	25.5	158	35.3	81	15.5
7	82	32.8	30	9.3	98	21.9	27	5.2
8	30	12.0	11	3.4	50	11.2	6	1.1
9	1	0.4	2	0.6	13	2.9	0	0
10	0	0	0	0	2	0.4	—	—
11	—	—	—	—	0	0	—	—
$\mathring{x}$	38.07±1.09		34.70±0.73		38.80±0.67		34.06±0.47	
a.v.	1.00		1.00		1		1	

TABLE 5. *Survival of flies from populations 1, 3, and 5*

	Population 1				Population 3				Population 5			
Weeks	♂♂	%	♀♀	%	♂♂	%	♀♀	%	♂♂	%	♀♀	%
0	479	100	432	100	449	100	385	100	435	100	483	100
1	440	91.9	416	96.3	425	94.7	375	97.4	415	95.4	451	93.4
2	389	81.2	395	91.4	375	83.5	356	92.5	361	83.0	424	87.8
3	345	72.0	385	89.1	318	70.8	340	88.3	285	65.5	384	79.5
4	289	60.3	345	79.9	281	62.6	318	82.6	238	54.7	346	71.6
5	209	43.6	312	72.2	231	51.4	274	71.2	191	43.9	306	63.4
6	115	24.0	254	58.8	183	40.8	186	48.3	136	31.3	251	52.0
7	81	16.9	185	42.8	140	31.2	95	24.7	92	21.1	189	39.1
8	54	11.3	83	19.2	84	18.7	53	13.8	41	9.4	80	16.6
9	29	6.1	38	8.8	55	12.2	15	3.9	12	2.8	35	7.2
10	20	4.2	21	4.9	23	5.1	9	2.3	1	0.2	4	0.8
11	6	1.3	5	1.2	4	0.9	1	0.3	0	0	0	0
12	2	0.4	1	0.2	0	0	0	0	—	—	—	—
13	0	0	0	0	—	—	—	—	—	—	—	—
$\mathring{x}$	32.42±0.83		43.04±0.83		36.53±0.95		40.26±0.76		32.02±0.83		39.30±0.83	
a.v.	.91		1.07		1		1		.90		1.00	

The survival data are given in table 4, along with calculations of the mean longevities and estimates of the adaptive values based on these means. There were no significant differences between the mean longevities of the same sex in the two populations; males of both populations, however, had a significantly greater mean longevity than females. The absence of significant differences in mean longevity led to equal estimates of adaptive value. Both males and females of populaton 6 had initial death rates higher than those found in population 7, but by the fifth or sixth week the proportion of survivors of either sex in 6 exceeded that in 7. This seems to indicate a greater variability in longevity among individuals of population 6 than among those of 7, but because of the population density-longevity interrelationship mentioned above, this is not a necessary interpretation.

b. Populations 1, 3, and 5. The technique for studying longevity was modified in the second experiment. Between 35 and 50 flies were placed in each half-pint bottle, and the sexes were kept separate. The survivors were etherized and counted weekly, as before. The flies for the analyses were obtained from subcultures from sample 38 of populations 1, 3, and 5. At this time, populations 1 and 3 were 38 generations old. Population 5 had existed for 17 generations and had received a total dose of 34,000 r. The approximate frequencies of second-chromosome lethals in the three populations were: (1) 20%, (3) 15%, and (5) 35%.

The relative longevities of the sexes differed in this study (table 5). In the case of females, flies from 3 (the control) and 5 had equal mean longevities, whereas females from population 1 were distinctly longer-lived. Males from the control population, however, lived longer than those from either 1 or 5, and the latter were about equal in this respect. The data, then, lead to two contrasting estimates of adaptive values: for females, (1) 1.07, (3) 1, (5) 1.00; for males, (1) .91, (3) 1, (5) .90. It is interesting to note the pattern of longevity of population-5 females as compared with those of population 3. For five weeks there were distinctly fewer survivors in 5 than in 3, but during the next four weeks the situation reversed and 5 had the greater proportion of survivors—nearly as many as population 1. This implies that the individuals in population 5 varied more with respect to lon-

TABLE 6. *Survival of virgin females from populations 6 and 7 after 40 generations of irradiation*

	Population 6		Population 7	
Weeks	Females	%	Females	%
0	46	100	45	100
1	45	97.8	44	97.8
2	26	56.5	32	71.1
3	14	30.4	15	33.3
4	6	13.0	5	11.1
5	2	4.3	1	2.2
6	1	2.2	0	0
7	1	2.2	—	—
8	0	0	—	—
$\bar{x}$	17.09±1.25		16.96±1.07	
a.v.	1.00		1.	

gevity than individuals from the control population at that time—a situation similar to that noted above for populations 6 and 7. The fact that there were fewer flies in each culture in the present case minimizes the objection raised there concerning the effect of population density on longevity.

c. Populations 6 and 7. The third longevity study involved an entirely different technique. Vials were made from 1½-inch sections of glass tubing (⅜ inch ID). A plug of food approximately 1 cm. deep was placed in one end of each tube by pushing it into a Petri dish of cornmeal-agar-molasses-brewers' yeast medium. This end was then sealed with hot paraffin. Virgin females obtained from sample 61 of populations 6 and 7 were placed in these tubes, one per tube, and the open ends were plugged with cotton. The tubes were mounted on a cardboard cylinder with loops of rubberized thread. The cylinder was kept in a desiccator over a saturated solution of Na_2CO_3 (87% relative humidity) at 25° C. Deaths were recorded daily, although the survival data given in table 6 have been converted to weeks to make them comparable with tables 4 and 5.

Again, there was no significant difference between the longevities of flies from the two populations. Once more, there were more early deaths among the flies of population 6, but nevertheless a higher final percentage of survival among them in the present case; this situation was the result of extremely small numerical differences.

EGG HATCHABILITY AND LARVAL COMPETITION

In two experiments both the percentages of eggs that hatched and the viabilities of larvae under competition were determined; in two other experiments egg hatchability alone was studied. The experiments consisted in collecting eggs from flies obtained from population egg samples, and from "Muller-5" flies, on 1″ × 3″ glass slides covered with a film of yeasted medium. (Muller-5 flies are a special stock of *D. melanogaster* identifiable by the mutant genes w^a, apricot, an eye color, and *B*, Bar, an eye shape.) These slides were exposed overnight to 50–100 pairs of wild-type of M-5 flies in otherwise empty half-pint bottles. Egg hatching was measured by transferring several hundred eggs the next morning to small glass slides covered with a film of food and observing the number of unhatched eggs after 24 and 48 hours. Other eggs were transferred to small discs stamped into non-yeasted food on other glass slides; 150 eggs laid by population flies and 200 eggs laid by M-5 flies were placed on each disc. Each disc, with its 350 eggs, was carefully lowered into a 2″ × 1″ shell vial containing 5 cc. of cornmeal-agar-molasses-brewer's yeast

TABLE 7. *Hatchability of eggs taken from the populations at the sample times indicated*

Sample	Population	No. eggs	% hatched	Prob.	a.v.
41	6	200	76.5	>.001	.85
41	7	200	90.5		1.
44	1	575	89.0	.5	1.00
44	3	575	91.3		1.
45	6	300	88.0	.01	.93
45	7	300	95.0		1.
46	1	400	90.7	.5	1.00
46	3	466	91.2		1.

TABLE 8. *Relative viabilities of flies from experimental populations in competition with Muller-5 flies.* Differential hatchability (see table 7) is included in these data.

Sample	Pop.	Muller-5 eggs	Muller-5 adults	Population eggs	Population adults	a.v. M-5 =1	P.	Pop. a.v.
41	6	400	200	300	181	1.21	.002	.79
41	7	400	200	300	229	1.53		1.
44	1	1200	741	900	626	1.13	.10	1.00
44	3	1200	818	900	716	1.17		1.

medium. These vials were decidedly overcrowded during larval development.

In comparing populations for egg hatching, direct comparison must be limited to populations studied simultaneously in this respect; the importance of slight variations in experimental procedure discourages comparison of results obtained at different times. The data on larval competition were analyzed by assuming that the Muller-5 flies, within any one test, could be used as a standard for comparison. In this way, adaptive values were calculated for the population flies with relation to the adaptive value of unity ascribed to the Muller-5 flies; the populations in the test were then compared by determining the relation of these calculated adaptive values. Thus, if population A survived competition twice as well as the Muller-5 strain, and population B three times as well, then A would have an adaptive value .67 as large as large as that of B. The data on larval competition (table 8) include egg hatching, larval viability, and differential mortality of pupae; the effect of differential egg hatching can be removed because the relative hatchability is known.

Four analyses of egg hatching were made; two comparisons of population 1 with population 3 (samples 44 and 46), and two comparisons of 6 with 7 (samples 41 and 45). The data are given in table 7. In both tests involving 6 and 7, the eggs from population 6 had lower hatching rates than those from 7. The tests of populations 1 and 3 showed no significant differences in percentages of eggs hatched, although in both experiments 1 had a lower frequency of hatched eggs than 3.

Samples 41 and 44 (comparing population 6 and 7 and 1 and 3, respectively) were studied for larval competition. Table 8 summarizes the data for these experiments, giving the adaptive values of the populations with relation to the Muller-5 flies and to each other. Populations 6 and 7 differed significantly in their competitive ability; the data indicate a survival of individuals from population 6 only .79 that of individuals from population 7 under these particular conditions. This value includes the difference in egg hatching already mentioned,

TABLE 9. *Summary of the adaptive values estimated by various techniques*

Technique	Population 1	3	5	6	7
Genetic test	1.03	1	.93	.95	.98
Male fertility	1.01	1	.89	.90	1.00
Longevity					
Exp. a. ♂♂	—	—	—	.98	.98*
a. ♀♀	—	—	—	.98	.98*
b. ♂♂	.91	1	.90	—	—
b. ♀♀	1.07	1	1.00	—	—
c. ♀♀	—	—	—	.98	.98*
Egg hatchability					
Sample 41	—	—	—	.83	.98*
44	1.00	1	—	—	—
45	—	—	—	.91	.98*
46	1.00	1	—	—	—
Larval competition (inc. egg hatch.)					
Sample 41	—	—	—	.77	.98*
44	1.00	1	—	—	—

* Set at .98 to agree with genetic test and, consequently, to make the calculations for 6 comparable to that test.

and so the relative competitive abilities were actually about .93 to 1. In the case of populations 1 and 3 the test showed no significant difference, although 1 had a lower competitive ability than 3 if the data are taken at face value (no lower, however, than has already been noted in the data on egg hatching.)

Discussion

The experiments described above are interesting in two respects: because they deal with characteristics of individuals taken from populations exposed to radiation; and because they add to the information concerning estimations of adaptive values in general. (For convenient reference, the data are recapitulated in table 9.)

Inspection of table 9 shows that the estimates of adaptive value derived from different studies are not identical. The estimated values for population 1, for instance, are in three cases significantly higher than those for population 3, in one case significantly lower, and in others not detectably different. The analysis of longevity of females indicated that population 5 had an adaptive value equal to that of the control population, but three other types of analysis indicated lower values. In a study of *D. pseudoobscura* (Wallace, 1948) estimates were made of the adaptive values of carriers of "sex-ratio." The results were comparable to those reported here in that estimates given by different techniques varied; the series as a whole, however, left little doubt as to relative adaptive values possessed by individuals of the different genotypes. Despite the different indications given by the various techniques the results do not vary haphazardly. The estimates for populations 5, 6, and 7 are mostly lower than those for population 1, and it appears that the populations can be ranked in adaptive value as follows: $1 \geq 3 \geq 7 > 6 \geq 5$.

The experimental populations for which these estimates were made had been subjected to several different conditions of irradiation. In spite of this, it was extremely difficult to demonstrate clear-cut differences in adaptive values. Chronic irradiation had detectable deleterious effects on the adaptive values of 5 and 6—perhaps, also, on 7. At the time these tests were made, any deleterious effects of X-radiation on population 1 had apparently been adequately compensated for, because the tests indicated for this population an adaptive value equaling, or very likely surpassing, that of the control population. It seems likely that artificially induced genetic variants have been included in the adaptive fabric of this population.

In the interpretation of these results at least two considerations should be kept in mind that were omitted from the above account. What transformations are necessary to change estimates based on isolated studies into true values encountered within populations? Is each of a series of estimations an estimate of the over-all adaptive value, or an estimate of one facet of the real value? The lack of data bearing on these points makes discussion of them premature. Lewontin (personal communication), using a technique similar to the genetic test of these experiments, attempted to estimate the adaptive values of *D. pseudoobscura* carrying different third-chromosome inversions; but the attempt failed, even though earlier population data had indicated adaptive values for some genetic constitutions of less than .5.

Summary

The most direct method of estimating adaptive values of various genotypes is through observation of the rates of change in their relative frequencies in certain environments. When this method is inapplicable the adaptive values may be inferred from physiological studies. A situation of this sort, involving experimental populations of *D. melanogaster*, is described. A brief history of each population is reviewed in table 1. A summary of estimates of adaptive values ob-

tained by a variety of techniques is given in table 9.

Estimates obtained by the different methods vary. Nevertheless, an indication of the relative values emerges from the series as a whole. This indicates a decreased adaptive value resulting from chronic irradiation, and an improvement in adaptive value arising through the action of selection on either induced or spontaneous mutations.

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NOTES AND COMMENTS

HOW MANY SPECIES?

G. G. Simpson

How many species have occurred in the whole history of life? The answer can only be a guess, but even a reasonable guess has some interest. The number could be calculated from the following data: (a) the mean number of contemporaneous species throughout the history of life, (b) the mean duration for all species, and (c) the length of time since life began. None of these figures is known. Fairly reasonable guessing is possible.

(a). The number of species now living is unknown, but can be estimated better than the number at any other time. Two million is a reasonable estimate, perhaps on the conservative side. The number of recent species is probably far above the all-time average, which might be guessed as somewhere between 250,000 and 1,000,000.

(b). There are some, but few, good data on durations of species. The durations vary enormously within groups; average durations of different groups are quite different; and the averages have changed markedly through time. A guess as to an over-all average is even more poorly controlled than the other guesses here made. A guess of something between 500,000 and 5,000,000 years may not be completely unreasonable.

(c). The earth is now believed to be on the order of three thousand million years old. Surface conditions in which life might have been possible are known to have existed at least two thousand million years ago. Fossil algae at least one thousand million years old are known. The origin of life occurred at least 1,000,000,000 years ago and perhaps 2,000,000,000 years or so.

These guesses can be used as follows to calculate possible total numbers of species:

	Guesses leading to estimates that are:		
	Maximal	Medial	Minimal
a. Mean contemporaneous spp.	1,000,000	625,000	250,000
b. Mean durations of spp.	500,000	2,750,000	5,000,000
c. Time since life began	2,000,000,000	1,500,000,000	1,000,000,000
ac/b. Total species	4,000,000,000	341,000,000	50,000,000

The total number of species in the history of life may have been between 50 and 5,000 million. As a more restricted guess, it may have been on the order of 500 million.

SIGNIFICANCE OF VARIATION IN PTYCHODERA FLAVA

K. Pampapathi Rao

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Ptychodera flava Eschscholtz is known to be highly variable (Horst, 1932). The occurrence of a large number of varieties in limited areas like the Maldive and Laccadive archipelagoes (Punnett, 1903) and the Gulf of Mannar (Rao, 1951), of a species like *Ptychodera flava* raises the question whether such variation is a step toward the formation of new species or only adaptations attending (or enabling) the covering of new environments.

The variable characters include color, the body size and the relative length of the branchial region.

It is known that changes in the environment give rise to phenotypic variation while the genotype is not altered (Dobzhansky, 1950). This explains the great variability, while the specificity is preserved, and thus the inner continuity which is the basic property of the species is not lost. The capacity of a geno-

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type to survive the new surroundings depends on its pre-adaptational range (Goldschmidt, 1933), and such a range is great in highly plastic species (Haldane, 1938). That such a plasticity is met with in *Ptychodera flava* is evidenced by its phenotypic variability, as well as by the histological instability exemplified by the observation of phenomena like metaplasia attending the regeneration of lost parts (Rao, 1951). But the formation of varieties may not be the beginning of speciation, since the different varieties show mostly quantitative (thus phenotypic) differences like relative length, coloration etc. Hence, in spite of the plasticity of the species and corresponding phenotypic variability caused by changes in the environment, the basic genotypic specificity is preserved.

But variations like the occurrence of a single collar pore (Rao, 1951) cannot be explained as phenotypic variations. Such a feature has far reaching effects on the physiology of the organism. It appears to have no relation to the change in the environment. Hence, it is likely that such characters as these may lead out of the limits of the species. Darwin regarded varieties as incipient species. Although later workers like Bateson and Goldschmidt have held that the differences between species are qualitatively and quantitatively distinct from those of varieties, there is no evidence that closely related species differ in a manner qualitatively diverse from varieties (Haldane, 1938). If varieties differ in a few genes, species may differ in hundreds of genes. Thus a gradual accumulation of differences, though primarily of the nature of variations, may lead a variety along the path of speciation.

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THE DUSKY-FOOTED WOOD RAT [1]

W. J. Hamilton, Jr.

The Hastings Natural History Reservation in Monterey County, California, has been a proving ground for many young western naturalists. Under the able directorship of Dr. Jean M. Linsdale, many important studies have been conducted on the mammals and other vertebrates of the area. The present volume is the result of a decade of intensive research on *Neotoma fusicipes*, the individual rats that lived in a small area in successive generations. Live trapping enabled the observers to make week to week observations on many of the 580 marked individuals. The authors were concerned primarily with the affairs of individuals; the length of time these rats remained at certain houses, their reasons for moving, relations with other members of the family and the social structure of the species in a given area.

The volume is divided into several chapters, including habitat, houses, animal associates, behavior, food, reproduction, morphology, and population. Each of these subjects is divided into lesser categories. The study suggests that wood rat populations are more stable than those of most rodents. Breeding occurs throughout the year. Social relations among the animals show varying degrees of attraction, tolerance and intolerance. These relations presumably determine the spacing of the rats and the length of time they occupy a given house. The health of the individual likewise is a factor which governs important phases of population behavior by restricting the activities of certain rats or by removing them. These observations once more point out the desirability of studying marked animals in the field, a necessity if one wishes to fathom the pattern of reproduction, social life and seasonal movements as exhibited in the wild state.

As in Linsdale's "The California ground squirrel," the reviewer feels the minutae often obscure the overall picture. Presumably a

[1] Jean M. Linsdale and Lloyd P. Tevis, Jr. Univ. California Press. The dusky-footed wood rat. v–vii, 1–664. $7.50.

part of the study consists of undigested notes of the 21 observers that are credited with aid in the investigations. The discussion on reproduction, occupying more than 100 pages, repeatedly recites lengthy case histories of the condition of the vulva, mammae and external genitalia; not once in this lengthy discourse is the reader given any clue to gestation length, a feature of reproduction surely worthy of discussion. The many graphs and tables are seldom referred to in the text, but they do serve a useful function. Additional tables would have permitted a reduction in the case histories that occur so frequently in the text. The photographs of living animals are not designed as illustrations, they purportedly present specific features of the animal itself, whether these be age groups, young, etc. Structural characteristics are better indicated against a pure white background rather than a simulation of natural habitat.

The great detail tends to obscure and aggravate the reader who is searching for generalizations. It is the reviewer's opinion that the book might well have been reduced by half and still have contained the essentials of the study.

Linsdale and Tevis are both accomplished biologists. Their study is one of the most complete mammalian life histories yet to appear. It is thus all the more distressing that the reader must search with such effort to find and interpret the facts in which he may be most interested.

UNDERSTANDING HEREDITY [1]

BRUCE WALLACE

This text seems to be designed for use in "science-survey" courses or for study by non-biologists of college caliber. A careful reading of this book should give the reader an understanding of heredity and hereditary mechanisms equivalent to that which results from a one-semester, "beginners" course in genetics. The material is adequate—one can quote topics from the Table of Contents—and it is all carefully explained by an expert: variation, selection, strains, sex-cells, fertilization, maturation, Mendelianism, chromosomes, mutation, linkage, multiple factors, multiple alleles, and thumbnail sketches of a number of specialized topics. Furthermore, there are a section of problems, a glossary, and a list of books for further study. This all adds up to a neatly packaged account of the subject.

A complaint that may be registered concerns this very neatness—it is overdone. Professor Goldschmidt has unorthodox ideas concerning at least two subjects: the gene and the mechanism of evolution. No one will realize this from reading "Understanding heredity." Except for a phrase or two delineating "many geneticists" from a person (or persons) unnamed, there is no indication that scientific conflicts exist. Actually, an account of controversies would probably appeal to and excite many of the readers to whom this book is addressed.

A further example of over-simplification is the use of human mental or emotional characteristics in connection with elementary genetics. Specific reference may be made to page 14 where this statement is made: ". . . it is known that a tendency toward wanderlust, an unsteadiness and proneness to nomadism, is inheritable." An illustration is constructed based on this statement. Now, there may or may not be good evidence for the inheritance of wanderlust (the reviewer knows of one paper dealing with five individuals of three generations) but the fact remains that the definition of the character is so abstract, that the tendency is so subject to suppression or modification by a variety of environmental, physical, or other emotional factors and is so subtle in its final expression that most intelligent readers will seriously question the validity of the original hypothesis.

It is good to see this attempt made to teach genetics to those who, although mentally alert in most matters, are so woefully ignorant concerning those mechanisms whereby they, their friends, and their children have come to be as they are. However, it is sincerely hoped that these people will be sufficiently inspired by the glimpses of modern genetical research problems that they will look into the advanced texts listed so conveniently by the author.

[1] Richard B. Goldschmidt. Understanding heredity. An introduction to genetics. John Wiley and Sons, New York. i–ix; 1–228; figs. 1–49. $3.75.

NOTE ON "CROSSING EXPERIMENTS WITH *CULEX* STRAINS"

P. G. SHUTE

In the article by Hannes Laven entitled "Crossing experiments with Culex strains" (Evolution, Vol. V., No. 4), on page 370, discussing the *A. maculipennis* complex in Europe, he states that "up till now their differentiation could be made only on the basis of markings of the eggs. Lately, however, morphological differences for the adults have been found for the two palaeartic species *A. atroparvus* and *A. messeae* in the shape of the wing scales (Laven, 1950)." In science, as in other walks of life, credit should be given where credit is due and therefore I should like to point out that it was Professor Ungureanu, a young Roumanian biologist who first discovered the differences in the shape and size of the plume scales of the wings of the *A. maculipennis* complex. On hearing of Ungureanu's observations in 1947 I was able to arrange for him to come to England and work on our indigenous *A. maculipennis*. This he did in 1948 and his results were published in 1948, proving that the differences in the shape of the wing scales of the English material was identical with the Roumanian material. These findings meant that for the first time hibernating female *A. maculipennis* could be identified according to species or varieties in the absence of eggs or male adults.

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